CORSO INTEGRATO DI GENETICA

AA 2011/12

Prof Alberto Turco

9.11.11 e 17.11.11

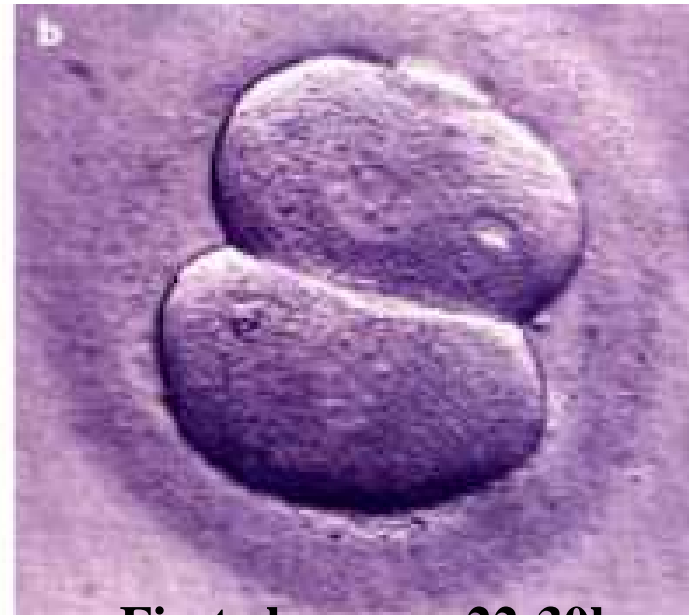
Lezioni 29 e 30

Lezioni 33 e 34

Cellule Staminali e

Medicina Rigenerativa

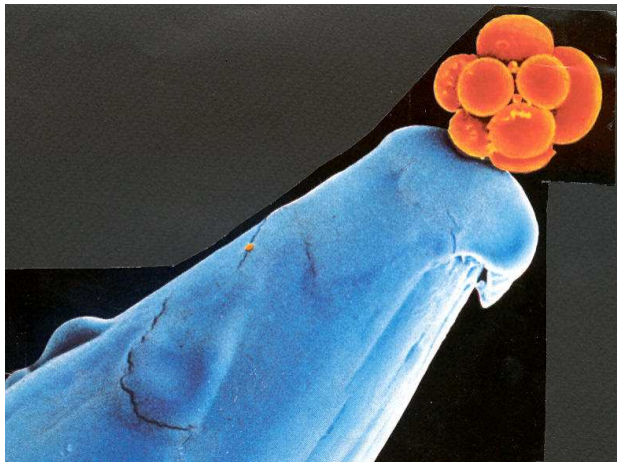
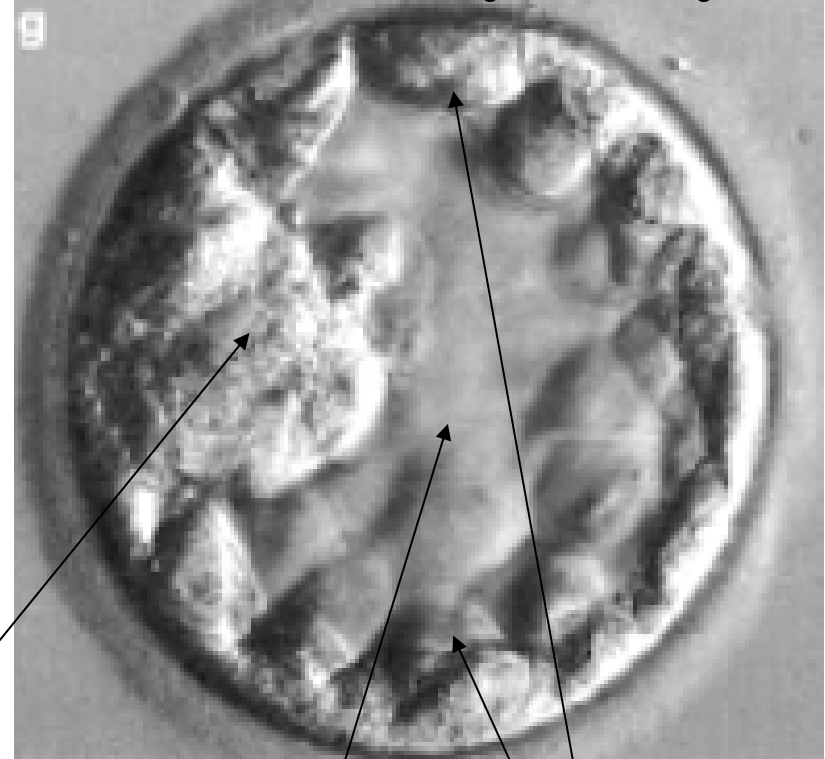
Early human preimplantation development in vitro

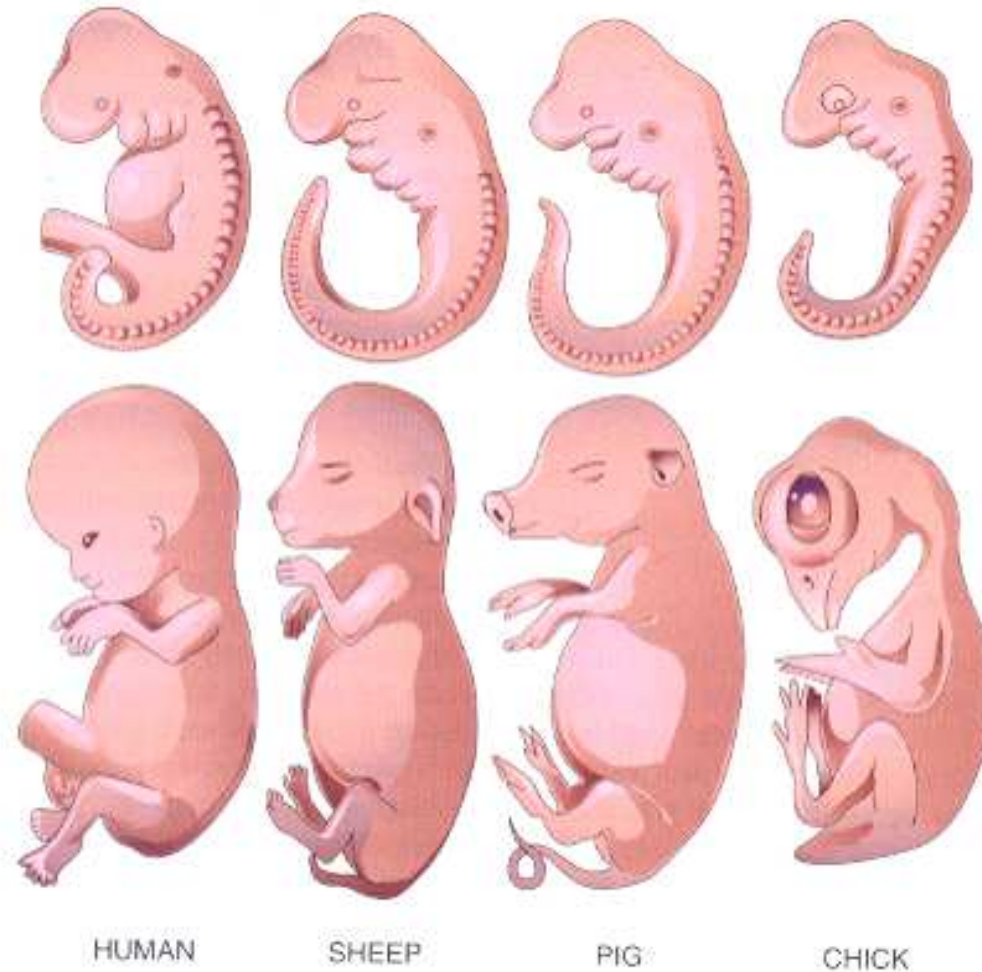
Pronuclear-stage embryo

First cleavage: 22-30h

4-cell stage

8-cell stage

Compaction, 16-32 cells

Human morula

Human blastocyst (day4-5)

Different early embryonic species resemble each other

(large head, pharyngeal arches, and tail)

Gli stadi embrionali rivelano uno stretto rapporto evolutivo tra i vertebrati (sorprendenti analogie anatomiche = discendenza da antenati comuni, che possedevano geni preposti allo sviluppo di branchie e code…..)

ZIGOTE
Ovocita fecondato

MORULA
8-16 cellule libere tra loro (blastomeri), 2-4 giorni post fertilizzazione

BLASTOCISTI
Circa 100 cellule, 5-6 giorni post fertilizzazione – Poche cellule **(ESC) sono ancora immortali** (3-4). Termina la fase pre-impianto: annidamento (inizio della gravidanza clinica)

PRE-EMBRIONE
Giorni 0-14 post-fertilizzazione: cellule totipotenti, manca individualità (gemellazione)

EMBRIONE
Dal giorno 14 sino a fine 8a settimana. Tutti gli abbozzi di organi e tessuti sono iniziati (organogenesi). Solo cuore e circolazione funzionano; dita palmate, coda tronca

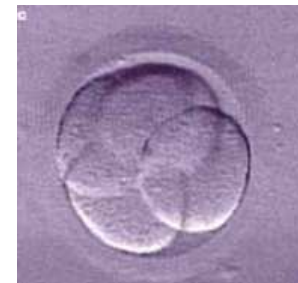
FETO
Dall'inizio della 9a s.g. alla nascita
Rapida differenziazione e crescita di organi (che diventano funzionanti);
crescita e aumento di peso

CELLULE STAMINALI
totipotenti

- Fino a **3-4 GIORNI DOPO FECONDAZIONE**
  (zigote e blastomeri della morula)

- può dare origine ad un individuo completo:
  CAPACITÀ MORFOGENETICA

- illimitata capacità moltiplicativa e proliferativa:
  IMMORTALITÀ CELLULARE

- può differenziarsi in tutti i tipi cellulari:
  CAPACITÀ DIFFERENZIATIVA

CELLULE STAMINALI pluripotenti

- da **4** a **6 GIORNI DALLA FECONDAZIONE** (cellule staminali **embrionali: blastocisti**)

- **NON PIU’ CAPACE** di dare origine ad un individuo completo: capacità morfogenetica **PERDUTA**

- illimitata capacità moltiplicativa e proliferativa: immortalità cellulare **MANTENUTA**

- può differenziarsi in tutti i tipi cellulari: capacità differenziativa **MANTENUTA**

CELLULE STAMINALI
multipotenti (adulte o somatiche)

- da ~**10** a **16 GIORNI DALLA FECONDAZIONE**
  (cellule staminali fetali, neonatali, del cordone, adulte)

- **NON PIU' CAPACE** di dare origine ad un individuo completo: capacità morfogenetica **PERDUTA**

- **NON PIU' CAPACE** di illimitata capacità moltiplicativa e proliferativa: immortalità cellulare **PERDUTA**

- può differenziarsi in tutti i tipi cellulari:
  capacità differenziativa **MANTENUTA**

CELLULE STAMINALI
l'origine

- **C.S. EMBRIONALI**
- **C.S. FETALI (?)** **da liquido amniotico (Jan 2007)**
- **C.S. "ADULTE" (SOMATICHE)**

(neonatali, cordonali, da tessuti ecc.)

STAMINALI DA LIQUIDO AMNIOTICO…

IL CASO

Repubblica 8.1.07

Importante scoperta degli scienziati Usa: potrebbe risolvere i dubbi etici sull'uso delle cellule

Staminali nel liquido amniotico

Nessun passeggero lo difende sotto accusa anche l'autista

Disabile sventa rapina picchiato sull'autobus

FEDERICA ANGELI

A PAGINA 26

UNA NUOVA fonte di staminali è stata trovata nel liquido amniotico. La scoperta – di fondamentale importanza per la scienza e l'etica – è stata fatta da un gruppo di scienziati italiani e americani. Si apre così una nuova strada per ottenere cellule utili alla cura di molteplici malattie senza bisogno di ricorrere agli embrioni umani. Le staminali, coltivate in laboratorio, si sono trasformate in vari tipi di tessuti: dai muscoli alle ossa, dal fegato al sangue. Per laici e cattolici, la scoperta fa sperare che le terapie siano ora molto più vicine.

DIENA e DUSI

ALLE PAGINE 14 e 15

Il Los Angeles Times accusa "Investe nelle società peggiori"

"Bill Gates un benefattore che fa solo del male"

VITTORIO ZUCCONI

A PAGINA 21

La scoperta degli scienziati

Annuncio dagli Usa: cellule staminali nel liquido amniotico

MILANO — Nel liquido amniotico si trovano cellule staminali embrionali. La scoperta, dovuta a scienziati dell'università di Harvard e di Wake Forest, potrebbe far superare i conflitti etici sull'uso delle staminali. ■ A pagina 19 **Pappagallo**

LE CONSEGUENZE PER L'ETICA

di **EDOARDO BONCINELLI**

Cellule staminali dal liquido amniotico? Sembra proprio l'uovo di Colombo. Tutti sapevamo che nel liquido amniotico ci sono molte cellule dello stesso tipo di quelle dell' embrione e l'analisi genetica eseguita ormai quasi di routine dopo l'amniocentesi utilizza proprio queste cellule per verificare l'assetto cromosomico e genetico del nascituro.

CONTINUA A PAGINA 19

CdS 8.1.07

Received 27 July 2006; accepted 20 November 2006; published online 7 January 2007; doi:10.1038/nbt1274

NATURE BIOTECHNOLOGY ADVANCE ONLINE PUBLICATION

Isolation of amniotic stem cell lines with potential for therapy

Paolo De Coppi[1,3], Georg Bartsch, Jr[1,3], M Minhaj Siddiqui[1], Tao Xu[1], Cesar C Santos[1], Laura Perin[1], Gustavo Mostoslavsky[2], Angéline C Serre[2], Evan Y Snyder[2], James J Yoo[1], Mark E Furth[1], Shay Soker[1] & Anthony Atala[1]

Stem cells capable of differentiating to multiple lineages may be valuable for therapy. We report the isolation of human and rodent amniotic fluid–derived stem (AFS) cells that express embryonic and adult stem cell markers. Undifferentiated AFS cells expand extensively without feeders, double in 36 h and are not tumorigenic. Lines maintained for over 250 population doublings retained long telomeres and a normal karyotype. AFS cells are broadly multipotent. Clonal human lines verified by retroviral marking were induced to differentiate into cell types representing each embryonic germ layer, including cells of adipogenic, osteogenic, myogenic, endothelial, neuronal and hepatic lineages. Examples of differentiated cells derived from human AFS cells and displaying specialized functions include neuronal lineage cells secreting the neurotransmitter L-glutamate or expressing G-protein-gated inwardly rectifying potassium channels, hepatic lineage cells producing urea, and osteogenic lineage cells forming tissue-engineered bone.

CELLULE STAMINALI DA LIQUIDO AMNIOTICO

Sembra abbiano proprietà intermedie tra CSE e CSA (necessari ulteriori studi)

- Questi risultati devono comunque essere riconfermati e riprodotti sperimentalmente

- Non sono un'alternativa o un sostituto alle CSE (le uniche vere pluripotenti)

- Rischio di perdita fetale da amniocentesi = 1%

Il professor Angelo Vescovi

Gli americani hanno ottenuto questo risultato da prelievi routinari, senza rischi né per la donna né per il feto

DA DOVE DERIVARE LE CELLULE STAMINALI ?

- **EMBRIONI (BLASTOCISTI) DOPO F.I.V.**

- **EMBRIONI DOPO S.C.N.T. NB: fonte non fisiologica (TRAPIANTO NUCLEARE)**

- **FETI ABORTITI**

- **SANGUE OMBELICALE ALLA NASCITA**

- **TESSUTI DELL'ADULTO**

Cellule Staminali

da ***stamen***, filo della vita

Sono cellule il cui destino non è ancora deciso…

indifferenziate, in grado di automantenersi illimitatamente

in grado di originare tutti i tipi di cellule diverse e mature dell’organismo (>200), attraverso un processo denominato differenziamento

in grado di comparire sui media prima che su riviste scientifiche……..

IL SERENO DIBATTITO SULLE CELLULE STAMINALI…..

Cellule staminali: breve cronistoria

-1961:“scoperte”le cs emopoietiche (ematologi)

-1981: isolate cse (ES) di topo

-1986: gene targeting su cellule ES (topi KO) (vd Nobel 2007 M.Capecchi)

-1998: prime linee cellulari di cse umane (hESC) (Thomson)

-1998: identificate cs nel cervello umano

-2006: iPS (induced pluripotent stem cells) nel topo
-2007: iPS nell’uomo (vettori virali)

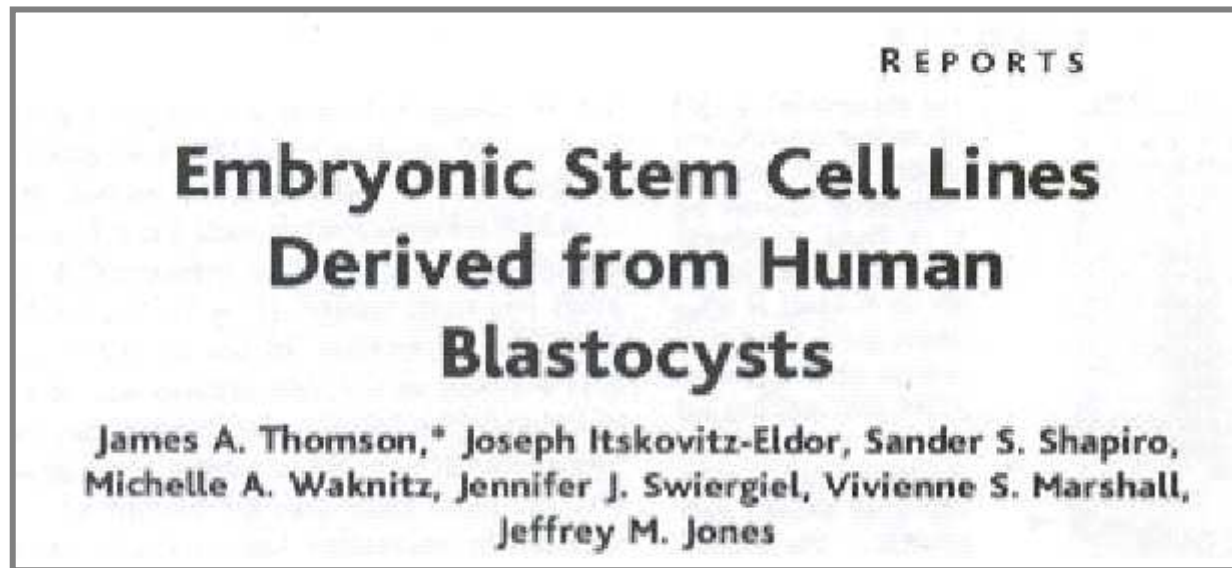
-2008: Science: “Reprogramming Cells” Breakthrough of the year

-2009: “virus-free” iPS cells nell’uomo

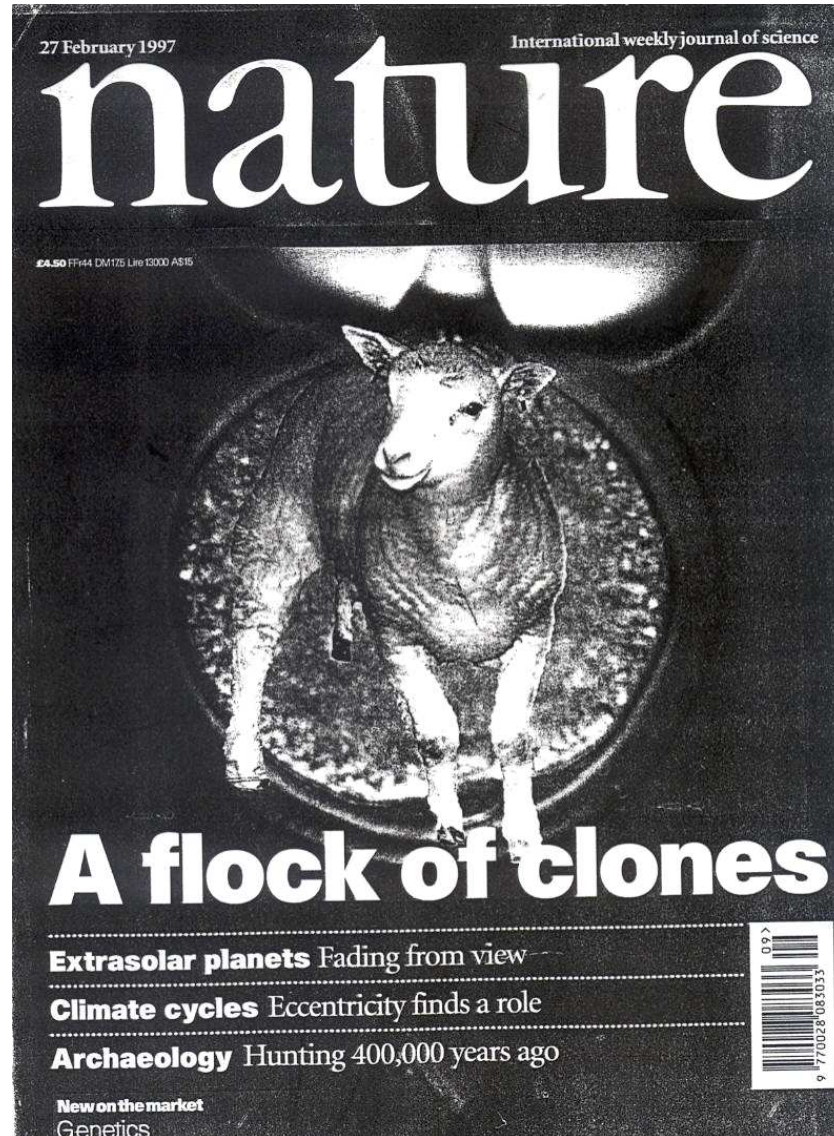
LA PRIMA VOLTA DELLE CELLULE STAMINALI EMBRIONALI UMANE…..

REPORTS

Embryonic Stem Cell Lines Derived from Human Blastocysts

James A. Thomson,* Joseph Itskovitz-Eldor, Sander S. Shapiro, Michelle A. Waknitz, Jennifer J. Swiergiel, Vivienne S. Marshall, Jeffrey M. Jones

Science, 1998

1997.........Dolly!!!

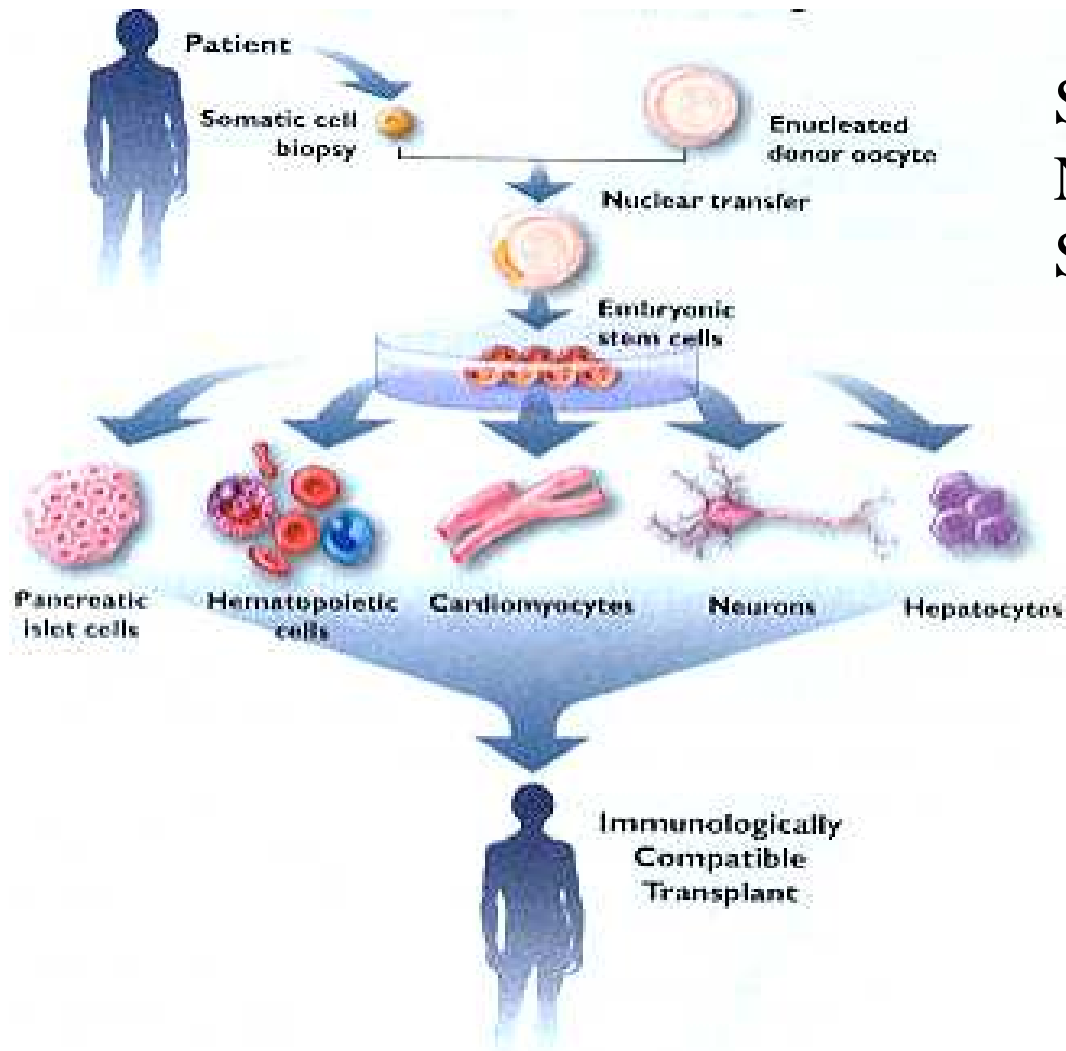
La pecora più famosa della storia...

Viable offspring derived from fetal and adult mammalian cells

I. Wilmut, A. E. Schnieke*, J. McWhir, A. J. Kind* & K. H. S. Campbell

Roslin Institute (Edinburgh), Roslin, Midlothian EH25 9PS, UK
* *PPL Therapeutics, Roslin, Midlothian EH25 9PP, UK*

Fertilization of mammalian eggs is followed by successive cell divisions and progressive differentiation, first into the early embryo and subsequently into all of the cell types that make up the adult animal. Transfer of a single nucleus at a specific stage of development, to an enucleated unfertilized egg, provided an opportunity to investigate whether cellular differentiation to that stage involved irreversible genetic modification. The first offspring to develop from a differentiated cell were born after nuclear transfer from an embryo-derived cell line that had been induced to become quiescent[1]. Using the same procedure, we now report the birth of live lambs from three new cell populations established from adult mammary gland, fetus and embryo. The fact that a lamb was derived from an adult cell confirms that differentiation of that cell did not involve the irreversible modification of genetic material required for development to term. The birth of lambs from differentiated fetal and adult cells also reinforces previous speculation[1,2] that by inducing donor cells to become quiescent it will be possible to obtain normal development from a wide variety of differentiated cells.

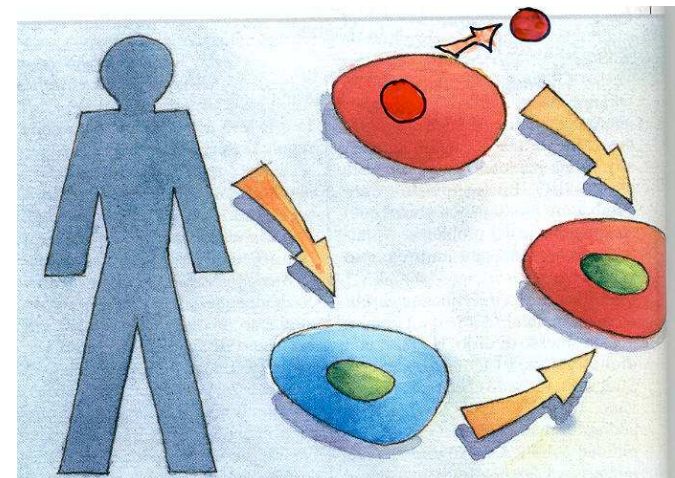
It has long been known that in amphibians, nuclei transferred from adult keratinocytes established in culture support development to the juvenile, tadpole stage[3]. Although this involves differentiation into complex tissues and organs, no development to the adult stage was reported, leaving open the question of whether a differentiated adult nucleus can be fully reprogrammed. Previously we reported the birth of live lambs after nuclear transfer from cultured embryonic cells that had been induced into quiescence. We suggested that inducing the donor cell to exit the growth phase

LA CLONAZIONE TERAPEUTICA NELL'UOMO

Somatic Cell
Nuclear Transfer
SCNT

Nature Medicine, 1999

CELLULE STAMINALI embrionali

- **ETEROLOGHE**
  da embrioni congelati sovrannumerari
  (NON immunocompatibili)

- **AUTOLOGHE**
  dopo trapianto nucleare somatico (clonazione terapeutica)
  (immunocompatibili)

C’era una volta……………………

Il punto d'avvio dell'epopea delle staminali adulte va fatto risalire al 1999, quando viene pubblicato su Science un articolo dal titolo promettente per la ricerca sulle cellule staminali: "Trasformare il cervello in sangue: un destino ematopoietico per le cellule staminali neuronali adulte in vivo". Autore di riferimento: **Angelo Vescovi**, del San Raffaele di Milano, e testimonial pro astensione nella campagna referendaria.

Il lavoro racconta di un esperimento effettuato sui topi: cellule staminali del cervello trapiantate in un topo irradiato (per ucciderne le cellule del sangue e favorire l'attecchimento di nuove cellule) si trasformano in linfociti B, linfociti T e cellule mieloidi in grande quantità, anche fino al 30%. La capacità di "ripopolare" il tessuto danneggiato è simile a quella ottenuta dopo trapianto con cellule del midollo. Questo risultato suggerisce che cellule staminali adulte (del cervello) hanno la capacità di transdifferenziare, in altre parole di produrre cellule d'altri tessuti (del sangue).

Una notizia rivoluzionaria perché smentisce un dogma fondamentale dell'embriologia: durante lo sviluppo dell'embrione si formano tre foglietti, da ciascuno dei quali poi si produrranno cellule con destini molto diversi. Non era mai accaduto che una cellula prodotta da un foglietto "saltasse" il confine embrionale, per produrne una di un tessuto d'origine diversa.

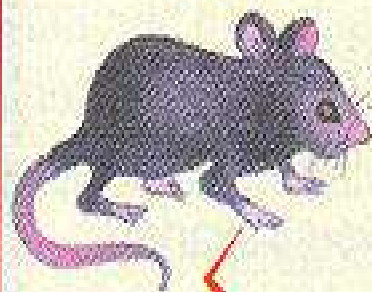
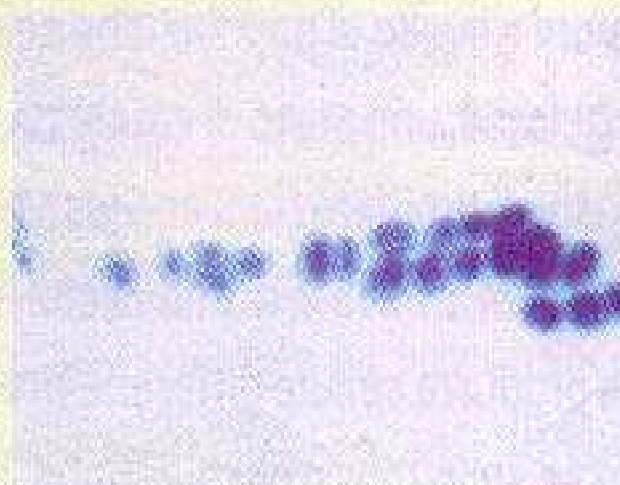
LE STAMINALI ADULTE TRANSDIFFERENZIANO?

Turning Brain into Blood: A Hematopoietic Fate Adopted by Adult Neural Stem Cells in Vivo

Christopher R. R. Bjornson,*†‡ Rodney L. Rietze,*§
Brent A. Reynolds, M. Cristina Magli, Angelo L. Vescovi‡

Gennaio 1999 Vol. 283 SCIENCE

Cellule del SNC diventano cellule del sangue (?)

Irraggiamento lesione ematopoietica
Cellule staminali cerebrali (blu)
Conversione di cellule cerebrali in cellule sanguigne
Analisi
Lesione muscolare
Cellule staminali cerebrali (blu)
Conversione di cellule cerebrali in cellule muscolari

…..Diversi laboratori tentano, senza successo, di ripetere l'esperimento di AV e nel febbraio 2002 su Nature Medicine il gruppo di Derek van der Kooy lo smentisce sostenendo che la transdifferenziazione, se esiste, è una proprietà assai rara. Infatti nel ripetere gli esperimenti pubblicati su Science non si ottengono i risultati di Vescovi.

Le ipotesi sono che il lavoro fosse inficiato da artefatti tecnici, oppure da caratteristiche particolari acquisite dalle cellule usate nel primo esperimento e non presenti in quelle usate dal gruppo di van der Kooy. Infatti i due gruppi, come molti altri, definiscono "cellule staminali del cervello" quella che in realtà è una neurosfera, una massa eterogenea di cellule nella quale non è chiaro se e quante staminali vi siano, né quale sia la loro reale natura……..

È chiaro solo che queste cellule sono instabili nel tempo, spesso finiscono con produrre soprattutto glia e non neuroni quando sottoposte a protocolli di differenziamento verso il tessuto nervoso.

Nat Medicine March 2002

Hematopoietic competence is a rare property of neural stem cells that may depend on genetic and epigenetic alterations

CINDI M. MORSHEAD[1], PATRICIA BENVENISTE[3], NORMAN N. ISCOVE[3] & DEREK VAN DER KOOY[2]

[1]Department of Surgery, [2]Department of Anatomy and Cell Biology, [3]The Ontario Cancer Institute and Department of Medical Biophysics, University of Toronto, Toronto, Ontario, Canada
N.N.I. and D.v.d.K. contributed equally to this study.
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The concept of stem-cell plasticity received strong support from a recent observation that extensively passaged, clonally derived neural stem cells could contribute to hematopoiesis. We investigated whether hematopoietic potential was a consistent or unusual feature of neural stem cells, and whether it depended on the extent of *in vitro* passaging before transplantation. Here we transplanted over 128 × 10^6 neurosphere cells into 128 host animals; however, we never observed contribution to hematopoiesis, irrespective of the number of passages and despite the use of an assay that could detect the contribution of a single blood stem cell to hematopoietic repopulation. Although extensively cultured neurosphere cells continued to generate neural progeny, marked changes in their growth properties occurred, including changes in growth-factor dependence, cell-cycle kinetics, cell adhesion and gene expression. Our results exclude hematopoietic competence as a consistent property of intravenously infused neural stem cells. However, the consistent changes that occurred during extended passaging are compatible with genetic or epigenetic alterations and suggest that rare transformation events may account for the neural-to-blood fate switch originally reported.

Stem cells in adult tissues, while generally displaying the capacity to differentiate into multiple cell types, have been thought to be restricted to generating cell types found in their tissue of origin. However, there is increasing evidence that neural stem cells can show plasticity and have the ability to generate cells of all of the germ layers[1–3]. There are indications that stem-cell populations depended on extended passage (up to 35 times) of the neural stem cells prior to transplantation, and possibly on accompanying genetic or epigenetic change[12–15]. This hypothesis predicted that neural stem-cell growth properties would observably alter with passage, but that hematopoietic capacity would only rarely be seen because the necessary specific genetic alterations could only

………Tra i due gruppi si sviluppa
una polemica sulle tecniche,
pubblicata su
Nature Medicine nel
giugno del 2002.

Hematopoietic potential of neural stem cells

To the editor—In the February 2002 issue of *Nature Medicine*, Morshead *et al.*[1] report the inability to generate hematopoietic progeny from neural stem cells (NSCs), raising several issues that must be addressed to avoid misinterpretation. Specifically, although they were purported to be identical, a number of substantial deviations exist between this study and our experiments showing the neurohematopoietic potential of NSCs (ref. 2). The functional characteristics of the NSC cultures described by Morshead *et al.* show that the cells that they used are unlike any known NSC, particularly those used in our transdifferentiation experiments[2]. These authors' cultures become consistently transformed, a property that we and others have never observed. Neither human nor mouse NSCs undergo transformation with passaging, but rather exhibit growth factor dependency, unaltered growth kinetics and prompt differentiation upon growth factor withdrawal over time[3-6]. Furthermore, Morshead *et al.* report that "less than 1% of the cells composing an individual neurosphere are NSCs," which is far below the current standard in this system: from 8% (postnatal) to over 20% (embryonic)[5-6]. Thus, Morshead *et al.* injected at least 20 times fewer NSCs than in our study, using populations of NSCs that displayed altered growth and differentiation capacity. Moreover, as noted by these authors, "transformed, aggressively growing cells would progressively eliminate non-transformed cells," further exacerbating their NSC deficiency. Eventually, the combination of low NSC number and significant transformation found in the Morshead *et al.* cultures would lead to the transplantation of a negligible number of NSCs. These authors' use of a high sensitivity method to detect hematopoietic engraftment cannot possibly compensate for such severe deficiency, particularly since the kinetic parameters that apply to engraftment in standard repopulation experiments with blood stem cells cannot be extrapolated to the different phenomenon that is the object of these studies, namely the expression of an hematopoietic fate by NSCs.

So far, neuro–hematopoietic conversion has been reported by three independent groups[2,7,8]. In particular, Shih *et al.* confirmed the work by Bjornson *et al.* by using human NSCs (ref. 8). Although Morshead *et al.* suggest these results were due to hematopoietic contamination, closer examination of the methods employed by Shih *et al.* rules out this possibility. Furthermore, additional studies have reported that NSCs transdifferentiate into non-hematopoietic mesodermal derivatives[9]. Thus, the ability of NSCs to give rise to non- neural cells should be less of a question, particularly in light of the variety of freshly-isolated and cultured somatic stem-cell types which appear capable of transdifferentiation[10].

We suggest that a more parsimonious conclusion should be drawn from the study of Morshead *et al.*, and submit that transdifferentiation experiments using transformed/transforming cultures with a negligible content of NSCs would necessarily yield an experimental outcome different than one using normal, highly clonogenic NSC cultures. The failure to detect transdifferentiation even at the single cell level is therefore not surprising. While we concur that hematopoietic transdifferentiation may represent a rare property of NSCs, we suggest that owing to disparate culture conditions and an unexplained NSC deficiency in the Morshead *et al.* experiments, it is inappropriate to compare this study to that of Bjornson *et al.*

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To the editor—Three independent groups have previously reported that NSCs are capable of differentiating into hematopoietic cells[2,7,8]. In disagreement with these previous studies, Morshead *et al.*[1] report that hematopoietic competence is not a propensity of cultured NSCs, but a rare property of NSCs that may depend on genetic and epigenetic alterations. Is there a possible explanation to reconciling the differences between the three previous reports and the report by Morshead *et al.*? Using the SCID-hu mouse model, we have reported that cultured human NSCs possess *in vivo* hematopoietic potential[8]. Although neurospheres from bulk cultures were used, extensive analyses, including a stromal-coculture system[11,12] that can detect the presence of a single hematopoietic cell, were performed to rule out the possibility of contaminating hematopoietic cells in our NSC cultures. Our results demonstrate that cultured human neurosphere cells commit and differentiate into hematopoietic stem cells (HSCs) in intact human bone marrow in SCID-hu mice. We estimate that one in a hundred cultured human NSCs are capable of differentiating into HSCs, which are responsible for initiating hematopoietic reconstitution in secondary recipients. Several hundred individual neurospheres have been analyzed in our laboratory for their potential to differentiate into neural progeny *in vitro*, and we have never observed a single individual neurosphere that has lost its ability to generate neural progeny *in vitro*. These results suggest that cultured human NSCs that possess hematopoietic potential *in vivo* have also maintained their NSCs ability to generate neural progeny *in vitro*, and that they represent a totipotent neurohematopoietic stem-cell population in human brain tissues.

As the frequency of NSCs in human brain tissues is about 0.5–1%, we estimate that the frequency for the totipotent neurohematopoietic stem-cell population in human brain tissues is about 1 in $1–2 \times 10^4$. Our data correlate well with the 1982 study of Bartlett *et al.*[7] that showed neurohematopoietic stem-cell population in adult mouse brain at the same frequency. In a recent analysis of muscle differentiation potential of NSCs by Galli *et al.*[9], all of the clones tested were myogenic and one of the clones, 2H1, was the same used earlier by Bjornson *et al.* in the hematopoietic study[2], showing a multipotential for neural, hematopoietic and myogenic potentials. Analyzing blastocyst chimeras in chick and mouse embryos generated using cultured neurospheres, Clarke *et al.*[13] have reported that NSC-derived cells were reproducibly found in various organs of the embryo derived from all three germ layers, with the exception of

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the hematopoietic system. Taken all together, these results suggest that cultured NSCs are heterogenous and there are multiple varieties of multipotent NSCs not only for neural cells but also for muscle, hematopoietic lineages, and cells in other tissues. It is distinctly possible that various multiple NSCs may exist in different brain regions or during various stages of development, and they may not be equally represented in different isolation techniques and/or culture conditions. It is clearly noticeable that the ≤1% cloning efficiency reported by Morshead *et al.* is significantly lower than the 7–20% reported by other groups[2,3,8,14] including ours, suggesting that the cultured NSCs used by Morshead *et al.* were different from the NSCs used by others, which might provide the basis for the difference in their ability to differentiate into hematopoietic lineages.

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THOMAS LEBON[3,4] &
STEPHEN J. FORMAN[1]
[1]*Division of Hematology/Bone Marrow Transplantation*
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[3]*Department of Molecular Biology Beckman Research Institute at the City of Hope*
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Morshead et al. *reply*—The two principal findings of our paper[1] were, first, that primary neural stem cell properties change with continued passaging *in vitro*, and second, that transdifferentiation of neural stem cells to hematopoietic cells is a rare occurrence. We note that Vescovi *et al.* concur in their letter that transdifferentiation is indeed a "rare property of neural stem cells." It is unfortunate that the insight was not highlighted more clearly in their original publication[2].

Both letters mention that other groups have now reported neurohematopoietic conversion. With rare exceptions, the published evidence for transdifferentiation has been obtained from populations rather than clones. We would argue that studies examining the transdifferentiation potential of cells will never be convincing unless they are done clonally. The original report from Bjornson *et al.*[2] was powerful because it did use clonally derived neural stem cell spheres as a starting population. However, we interpret their example of transdifferentiation to be the result of a rare genetic or epigenetic transformation that occurred in culture. Even some of the more frequent examples of transdifferentiation to muscle[9,15,16] are now suspect due to recent reports of cell fusion events[17,18]. Conclusive demonstration of multilineage potential will demand rigorous analysis at a clonal level.

"I don't know if neuronal stem cells can or cannot become blood cells but one thing is for sure, neuronal stem cells can become controversial."

Both letters suggest that our failure to replicate hematopoietic transdifferentiation is due to a difference in our starting neural stem cell populations. We reported that less than 1% of all the cells in a primary neurosphere are stem cells whereas other groups report a range of 5–19%[3,5,19]. The apparent discrepancy is easily explained by the technical differences in acquiring these values: our frequencies were based on total cell counts through cryosectioned spheres, while others based their estimates on viable cell counts after dissociation. Indeed, Gritti *et al.*[19] report an average of 50 new spheres from a single neurosphere dissociation which is actually less than our observed numbers of 80–100 new spheres, yet they conclude that 5% of all neurosphere cells are stem cells. Hence, the higher relative frequencies are a result of underestimating the starting population of cells within each neurosphere.

More significantly, we showed that neural stem cell frequencies within spheres increase with passage, and observed frequencies as high as 17% in spheres cultured to the extent used by Bjornson *et al.*[2]. However, even our extensively passaged spheres, containing stem cells at relatively high frequencies, failed to yield hematopoietic cells after injection into mice. Vescovi *et al.* propose that, "eventually, the combination of low NSC number and significant transformation found in the Morshead *et al.* cultures would lead to the transplantation of a negligible number of NSCs." This statement is incorrect. Even at stem-cell frequencies of 0.5%, mice transplanted with 1×10^6 cells received 5,000 neural stem cells each. We thoroughly tested extensively passaged cells as well. These were never observed to lose trilineage neural differentiation capacity, and mice injected with 1×10^6 cells received up to 170,000 neural stem cells each. Ultimately, we screened a total of 12 million neural stem cells without witnessing a single instance of hematopoietic reconstitution.

Given that the correspondents and ourselves are in agreement that hematopoietic transdifferentiation of neural stem cells is rare, the key remaining question is whether such rare events occur spontaneously or whether they depend on transformations in culture.

CINDI M. MORSHEAD[1],
DEREK VAN DER KOOY[2] &
NORMAN N. ISCOVE[3]

...Nel frattempo un altro articolo autorevole (Clarke et al., Science 2000) afferma che le stesse cellule proliferanti, estratte da cervello e trapiantate in una blastocisti di topo, contribuiscono a creare tutti i tessuti, **a eccezione di uno: il sangue**.

Proprio il tessuto che invece sarebbe stato prodotto nell'esperimento del 1999.....!!!

2 JUNE 2000 VOL 288 SCIENCE

Generalized Potential of Adult Neural Stem Cells

Diana L. Clarke,[1] Clas B. Johansson,[1,2] Johannes Wilbertz,[1] Biborka Veress,[1] Erik Nilsson,[1] Helena Karlström,[1] Urban Lendahl,[1] Jonas Frisén[1*]

The differentiation potential of stem cells in tissues of the adult has been thought to be limited to cell lineages present in the organ from which they were derived, but there is evidence that some stem cells may have a broader differentiation repertoire. We show here that neural stem cells from the adult mouse brain can contribute to the formation of chimeric chick and mouse embryos and give rise to cells of all germ layers. This demonstrates that an adult neural stem cell has a very broad developmental capacity and may potentially be used to generate a variety of cell types for transplantation in different diseases.

of these cells.

Although we reproducibly found neural stem cell progeny in various organs in chick and mouse embryos, other tissues contained no *lacZ*-expressing cells. For example, we did not detect any contribution to the hematopoietic system in the models we used. This

….Anche l'altra prestigiosa
rivista **Cell** (Krause et al. 2001),
descrivendo la (presunta) plasticità
delle HSC, <u>non</u> documenta la
"transdifferenziazione"
in tessuto nervoso…….

Cell, Vol. 105, 369–377, May 4, 2001, Copyright ©2001 by Cell Press

Multi-Organ, Multi-Lineage Engraftment by a Single Bone Marrow-Derived Stem Cell

Diane S. Krause,[1,5,6] Neil D. Theise,[3,6]
Michael I. Collector,[4] Octavian Henegariu,[2]
Sonya Hwang,[3] Rebekah Gardner,[3]
Sara Neutzel,[4] and Saul J. Sharkis[4]
[1]Department of Laboratory Medicine and
[2]Department of Genetics
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New Haven, Connecticut 06520
[3]Department of Pathology
New York University Medical School
New York, New York 10016
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Johns Hopkins School of Medicine
Baltimore, Maryland 21231

Summary

Purification of rare hematopoietic stem cell(s) (HSC) to homogeneity is required to study their self-renewal, differentiation, phenotype, and homing. Long-term repopulation (LTR) of irradiated hosts and serial transplantation to secondary hosts represent the gold standard for demonstrating self-renewal and differentiation, the defining properties of HSC. We show that rare cells that home to bone marrow can LTR primary and secondary recipients. During the homing, CD34 and SCA-1 expression increases uniquely on cells that home to marrow. These adult bone marrow cells have tremendous differentiative capacity as they can also differentiate into epithelial cells of the liver, lung, GI tract, and skin. This finding may contribute to clinical treatment of genetic disease or tissue repair.

← **NB: not brain!!**

….Ma nel 2002 Wagers et al. su Science sferrano il primo colpo contro queste speranze: smentiscono la possibilità di produrre neuroni con cellule staminali del sangue.

Nel lavoro si afferma che solo una rara cellula staminale donatrice sarebbe diventata un neurone del cervelletto….

…e questo si dimostrerà, poi, il risultato di una **fusione** cellulare e **non** il prodotto di una transdifferenziazione……

27 SEPTEMBER 2002 VOL 297 SCIENCE

Little Evidence for Developmental Plasticity of Adult Hematopoietic Stem Cells

Amy J. Wagers,* Richard I. Sherwood, Julie L. Christensen, Irving L. Weissman

To rigorously test the in vivo cell fate specificity of bone marrow (BM) hematopoietic stem cells (HSCs), we generated chimeric animals by transplantation of a single green fluorescent protein (GFP)–marked HSC into lethally irradiated nontransgenic recipients. Single HSCs robustly reconstituted peripheral blood leukocytes in these animals, but did not contribute appreciably to nonhematopoietic tissues, including brain, kidney, gut, liver, and muscle. Similarly, in $GFP^{+}:GFP^{-}$ parabiotic mice, we found substantial chimerism of hematopoietic but not nonhematopoietic cells. These data indicate that "transdifferentiation" of circulating HSCs and/or their progeny is an extremely rare event, if it occurs at all.

....Rassegna stampa sulla presunta transdifferenziazione (o "plasticità") delle cellule staminali adulte, <u>ripetutamente smentita</u>, e risultata invece una fusione cellulare <u>(ripetutamente confermata)</u>.....

LA PRESUNTA PLASTICITA'
DELLE CELLULE STAMINALI ADULTE

Stem cells

Cell fusion causes confusion

Aprile 2002, Nature

Andrew E. Wurmser and Fred H. Gage

'Transdifferentiation' is a poorly understood process invoked to explain how tissue-specific adult stem cells can generate cells of other tissues. New results challenge its existence.

Changing potency by spontaneous fusion

Qi-Long Ying*, Jennifer Nichols*, Edward P. Evans† & Austin G. Smith*

*Centre for Genome Research, University of Edinburgh, The King's Buildings, West Mains Road, Edinburgh EH9 3JQ, UK
† Department of Zoology, University of Oxford, South Parks Road, Oxford OX1 3PS, UK

Bone marrow cells adopt the phenotype of other cells by spontaneous cell fusion

Naohiro Terada*†, Takashi Hamazaki*, Masahiro Oka*, Masanori Hoki*, ... Laurence Morel*,

Haematopoietic stem cells do not transdifferentiate into cardiac myocytes in myocardial infarcts

Charles E. Murry[1], Mark H. Soonpaa[2], Hans Reinecke[1], Hidehiro Nakajima[2], Hisako O. Nak... Kishore B. S. Pasumarthi[2], Jitka ... Veronica Poppa[1], Gillian Bradford...

Haematopoietic stem cells adopt mature haematopoietic fates in ischaemic myocardium

...y J. Wagers[2,3], Julie L. Christensen[2,3], ... L. Weissman[2,3] & Robert C. Robbins[1]

Aprile 2004, Nature

FUSIONE CELLULARE E NON TRANSDIFFERENZIAZIONE
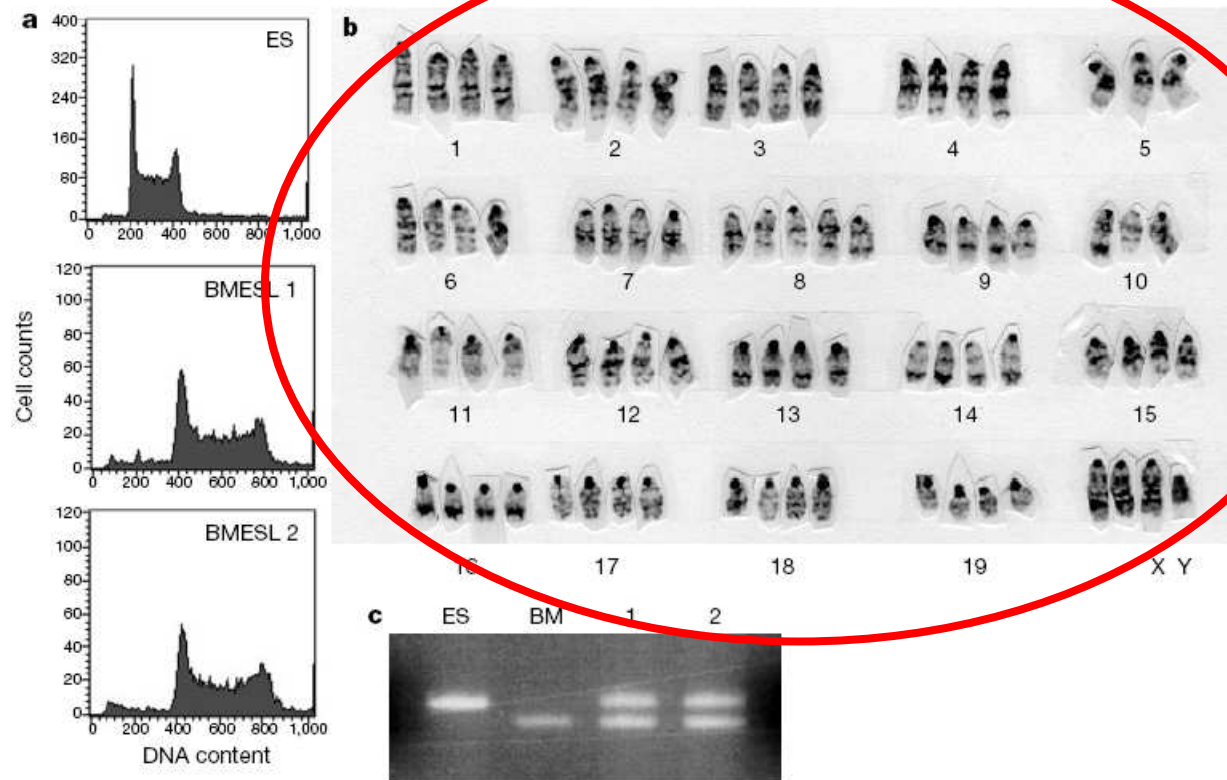
Figure 2 Genetic analysis of BMESL cells. **a**, DNA ploidy. Parental embryonic stem (ES) cells and BMESL cells (clone numbers 1 and 2, 4 weeks after cloning) were stained with propidium iodide and subjected to FACS analysis. **b**, Karyotype (BMESL clone 1) **c**, DNA polymorphism. Genomic DNA was extracted from embryonic stem cells, bone marrow (BM) cells, and BMESL cells (clones 1 and 2). DNA was amplified using microsatellite primers detecting polymorphisms between the bone marrow genome and the embryonic stem cell genome, separated on 5% agarose gel and visualized by ethidium bromide staining. Hybrid 129/B6 genotypes were detected in BMESL clones 1 and 2 for chromosomes 1 (D1MIT15), 9 (D9MIT48), 11 (D11MIT20), 14 (D14MIT11), 17 (D17MIT42) and 18 (D18MIT14).

LE STAMINALI ADULTE TRANSDIFFERENZIANO?

ARTICLES

Marzo 2002 Nat. Medicine

Hematopoietic competence is a rare property of neural stem cells that may depend on genetic and epigenetic alterations

CINDI M. MORSHEAD[1]

Little Evidence for Developmental Plasticity of Adult Hematopoietic Stem Cells

Amy J. Wagers,* Richard I. Sherwood, Julie L. Christensen, Irving L. Weissman

Settembre 2002 Vol. 297 SCIENCE

…se transdifferenziano è un fenomeno rarissimo

CARDIOLOGIA RIGENERATIVA?

Cardiac Cell Therapy — Mixed Results from Mixed Cells

Anthony Rosenzweig, M.D.

Despi
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Intracoronary Injection of Mononuclear Bone Marrow Cells in Acute Myocardial Infarction

Ketil Lunde, M.D., Svein Solheim, M.D., Svend Aakhus, M.D., Ph.D., Harald Arnesen, M.D., Ph.D.,
Michael Abdelnoor, Ph.D., Torstein Egeland, M.D., Ph.D., Knut Endresen, M.D., Ph.D., Arnfinn Ilebekk, M.D., Ph.D.,
Arild Mangschau, M.D., Ph.D., Jan C
Haakon Kiil Grøgaard, M.D., Re
Einar Hopp, M.D., Asgrimur Ragnarss

Intracoronary Bone Marrow–Derived Progenitor Cells in Acute Myocardial Infarction

Volker Schächinger, M.D., Sandra Erbs, M.D., Albrecht Elsässer, M.D.,
Werner Haberbosch, M.D., Rainer Hambrecht, M.D., Hans Hölschermann, M.D.,
Jiangtao Yu, M.D., Roberto Corti, M.D., Detlef G. Mathey, M.D.,
Christian W. Hamm, M.D., Tim Süselbeck, M.D., Birgit Assmus, M.D.,
Torsten Tonn, M.D., Stefanie Dimmeler, Ph.D., and Andreas M. Zeiher, M.D.,

Settembre 2006 N ENG J MED Vol.355 n12

…non ancora

LE CELLULE STAMINALI
E LA PLASTICITA'

TEMPO MEDICO **Giugno 2005**

Adulte e deludenti

Un caso eclatante di provincialismo scientifico. Gli esperimenti di transdifferenziazione delle staminali non hanno mantenuto le promesse. Solo in Italia si sostiene che le

La campagna referendaria è stata segnata dalla contrapposizione fra difensori delle staminali embrionali e crociati delle staminali adulte. Di fatto, oggi in Italia l'orientamento è di sperimentare solo sulle seconde, e i 7,5 milioni di euro destinati dalla Commissione nazionale sulle staminali riguardano essenzialmente le "adulte" e quelle derivate dal cordone ombelicale. Ma quanto è davvero fertile questo terreno di ricerca? E fino a che punto lo studio di queste può evitare il "sacrificio" delle blastocisti?

Il punto d'avvio dell'epopea delle staminali adulte va fatto risalire al 1999, quando viene pubblicato su Science un articolo dal titolo promettente per la ricerca sulle cellule staminali: "Trasformare il cervello in sangue: un destino ematopoietico per le cellule staminali neuronali adulte in vivo". Autore di riferimento: Angelo Vescovi, del San Raffaele di Milano, e testimonial pro astensione nella campagna referendaria.

Lettera Aperta al Presidente del Consiglio dei Ministri ***Romano Prodi***
dal Gruppo di ricercatori italiani sulle
cellule staminali embrionali

La ricerca sulle CSE non è un inutile optional………

Roma, 14 luglio 2006

Oggetto: ***Perché la ricerca sulle cellule staminali embrionali umane non e' un inutile "optional", ma è doverosa per il progresso della scienza ed è una pratica legalmente permessa in Italia.***

Egregio Presidente,
Noi ricercatori, che, in Italia, stiamo conducendo studi su linee di cellule staminali embrionali preparate all'estero, ci siamo riuniti in un Gruppo indipendente ed abbiamo organizzato per oggi un Convegno di studio a Roma per presentare all'opinione pubblica le nostre ricerche scientifiche e per sgombrare il campo circa la assoluta legittimità degli studi che stiamo facendo.

Le inviamo questa Lettera Aperta per informarLa dei principali risultati del nostro Convegno e per chiedere il sostegno del Governo e Suo alle nostre ricerche.

A questo proposito ribadiamo che:

- **queste ricerche sulle cellule staminali sono campo di frontiera, nuovo e ricco di prospettive, potenzialita' e quindi anche di speranze.** Esse contribuiranno all'avanzamento della conoscenza e allo studio delle malattie umane, con un continuo lavoro per alzare il livello di lotta alle patologie, con benefici per l'umanità tutta.

- **le ricerche sulle cellule staminali embrionali sono necessarie quanto quelle sulle staminali adulte.** Non esiste contrapposizione ma complementarità tra queste ricerche. Le scoperte sulle prime costantemente favoriscono gli studi sulle altre, e viceversa. Inoltre, a tutt'oggi, nessuna e' sinonimo di garanzia di cura per tutte le malattie umane. Per questo, a tutt'oggi, e' "scientificamente sbagliato" impedire che questa sinergia possa funzionare.

- **la percezione, da alcuni veicolata all'opinione pubblica, che le cellule staminali siano un "mero strumento di trapianto", e' frutto di una comunicazione superficiale e deviante.** Non c'e' niente di piu' sbagliato. Ad esempio, le cellule staminali embrionali presentano caratteristiche tali da renderle un preziosissimo elemento di conoscenza per giungere a capire lo sviluppo dei nostri tessuti, le molecole implicate o come si ammalino alcune delle nostre cellule. Non solo, possono essere usate per sviluppare e testare farmaci o per capire la tossicita' di composti dannosi alla salute del feto. Il trapianto cellulare rappresenta, quindi, soltanto uno dei potenziali ambiti applicativi delle cellule staminali, siano esse embrionali o adulte.

- **la "curiosa" campagna secondo cui la ricerca sulle staminali embrionali sarebbe finanziata da non bene identificate "lobby internazionali", attente solo all'aspetto economico è falsa, inconsistente e faziosa.** Al contrario, queste ricerche sono, per la quasi totalita', rigorosamente controllate e sostenute economicamente da Enti Pubblici e da Fondazioni.

- **l'affermazione secondo cui i finanziamenti per la ricerca sulle staminali embrionali "sottragga ingenti fondi" a quella sulle staminali adulte è altrettanto falsa.** Il Ministero della Ricerca ha già messo in evidenza che le staminali di origine embrionale compaiono in un numero esiguo di progetti Europei e che hanno ricevuto una frazione irrisoria del budget complessivo. All'atto pratico, i due campi si sostengono l'un l'altro, anche come possibilita' di accesso ai fondi per la ricerca. Non si tratta di due strade parallele ma di una rete di conoscenze che si intersecano. Dimostrazione e' che molti scienziati nel mondo, nei laboratori, lavorano sia sulle une che sulle altre.

- **tutti i ricercatori che lavorano solo sulle staminali adulte, devono avere l'onesta' scientifica e intellettuale di ricordare, sempre, a sé stessi, alla gestione politica e all'opinione pubblica quanto beneficino e beneficieranno delle ricerche sulle staminali embrionali.** Devono ricordare quanto traggono dal partecipare a progetti internazionali di ricerca che contemplano entrambi i tipi cellulari. E quanto i risultati ottenuti siano interdipendenti. Qualcuno, correttamente, lo fa. Qualcun altro invece no. Non farlo e' grave e distorcente nei confronti della societa' intera. Peggio ancora è alimentare il clima di sospetto e l'azione tesa a screditare la ricerca sulle staminali embrionali.

- **la ricerca sulle cellule staminali embrionali in Italia e' legale.** Sosteniamo inoltre che, anche dal punto di vista etico, le nostre ricerche sono pienamente legittime e doverose. Non è questa la sede per affrontare il tema dell'embrione, ma quello che è certo oltre ogni ragionevole dubbio è che una cellula staminale embrionale non è un embrione, e che lavorare su queste cellule non equivale affatto a lavorare su un embrione.

- **i nostri progetti di ricerca sono stati approvati da un Comitato etico indipendente che si è fatto garante della loro rilevanza scientifica e della legittimità dei finanziamenti, nonché dell'osservanza della normativa vigente (anche Regionale) e della consonanza all'etica.** Ci impegniamo a continuare questa prassi ed a rendere conto a Lei e all'opinione pubblica di quanto andiamo facendo – anticipando eventuali ricerche controverse.

- **la libertà di ricerca scientifica è principio sacrosanto accolto ed esplicitato nella nostra Costituzione.** Vorremmo che alle dichiarazioni soprattutto i fatti anche per quanto attiene al nostro settore di ricerca. Siamo preoccupati che la Carta fondamentale della nostra società sia violata non dai nostri studi, ma da chi tenta di limitare la libertà di ricerca sulla scorta di strumentali e ingiustificate interpretazioni restrittive alla già restrittiva Legge 40/2004.

Come scienziati **chiediamo che** alle nostre ricerche innovative sia dato il giusto rilievo, e siamo aperti a qualsiasi confronto trasparente e costruttivo. Siamo pronti e sempre disponibili a presentare in pubblico ciò che stiamo facendo, perché la scienza è un'attività che deve essere sempre svolta nella totale trasparenza e nel dialogo argomentato – senza pregiudiziali.

Signor Presidente, favorisca le nostre ricerche nelle forme a Lei possibili, perché queste ricerche sono parte significativa e fondamentale del bene comune: la salute di domani si garantisce soprattutto con le scelte di oggi. Un paese come l'Italia non può sottovalutare le nuove opportunità che si sono aperte sul piano scientifico in questo settore. È per questo che ci siamo rivolti direttamente a Lei sicuri di trovare sostegno.

Il Gruppo dei Ricercatori Italiani sulle cellule staminali embrionali

- ***Elena Cattaneo (Università di Milano)***
- ***Gianluigi Condorelli (I.R.C.C.S. Multimedica, Milano, Fondazione Parco Biomedico San Raffaele Roma)***
- ***Cesare Galli (LTR-CIZ, Spallanzani, Cremona, Università di Bologna)***
- ***Fulvio Gandolfi (Università di Milano)***
- ***Alessandro Mugelli (Università di Firenze)***
- ***Federica C. Sangiuolo (Università di Roma "Tor Vergata")***

G-2005

Il Sole 24 ORE
Quotidiano Milano Direttore: Ferruccio De Bortoli Lettori Audipress 1204000

Dallapiccola: non serve la ricerca sull'embrione

È un dato di fatto. Il dibattito politico sui referendum più nelle mani dei partiti politici, divisi peraltro in maniera trasversale sull'argomento, è in quelle dei "Comitati". L'ultimo in ordine di tempo, è quello che viene presentato ufficialmente oggi a Roma e che

24,2 per cento. Un piccolo calo, lo ammetto, ma che cosa abbiamo guadagnato nel frattempo? Innanzitutto, limitando a tre il numero di ovociti non si creano embrioni in eccesso, e non è poco visto che ci stiamo chiedendo cosa farne di quelli congelati. E poi, sul fronte della salute, la donna è più protetta: per ottenere come avveni-

LA CONOSCENZA NON SI PUO' FERMARE…
(la ricerca non si può bloccare su basi ideologiche)

…NICA 15 MAGGIO 2005 - N. 132 — PAGINA 35

APPELLO DI 77 NOBEL

Sì alla ricerca sulle staminali embrionali

Ai 77 Nobel che hanno firmato l'appello all'Onu pubblicato qui in esclusiva, andrebbero idealmente aggiunti i due italiani Renato Dulbecco e Rita Levi Montalcini, che insieme a Umberto Veronesi e a decine di altri scienziati italiani hanno sottoscritto un analogo documento italiano. Se ne discuterà il 18 maggio, alle 12 a Roma (via Nazionale 22), in un incontro organizzato da comitato «Ricerca e salute». L'appello dei Nobel è stato promosso dall'Associazione Luca Coscioni e dal Partito radicale transnazionale, e presentato presso la sede delle Nazioni Unite di New York insieme al Genetics Policy Institute, la Coalition for the Advancement of Medical Research e la Christopher Reeve Foundation. Molte le firme di biologi (Guillemin, Nuesslein-Volhard, Arber, Hartwell, Greengard, De Duve, Sulston, Cohen, Thomas, Benacerraf, Lauterbur, Blobel, Horvitz, Roberts, Baltimore, Varmus, Kornberg) ma anche di chimici, fisici, economisti (tra cui Kenneth Arrow) e del romanziere Josè Saramago.

Noi sottoscritti, cittadini di tutto il mondo, personalità della scienza, della cultura e della politica ci uniamo per dare corpo e voce a una speranza di vita e di salute che oggi passa per la libertà della ricerca scientifica e che rifiuta vecchi e nuovi proibizionismi anti-scientifici e ideologici.

Grazie al rapido progresso della ricerca

LA RICERCA DOVREBBE POTER PERCORRERE TUTTE LE STRADE POSSIBILI……….

23-APR-2005 CORRIERE DELLA SERA Quotidiano Milano Direttore: Paolo Mieli Lettori Audipress 2927000

«Usiamo gli embrioni congelati per la ricerca»

I Lincei sulle staminali oggetto del referendum sulla procreazione. Scontro tra scienziati: siete nazisti

GLI EMBRIONI E LA RICERCA

11-MAG-2005 Quotidiano Roma Direttore: Antonio Padellaro Lettori Audipress 409000

L'intervista Giulio Cossu

Istituto di ricerca staminali Milano

«Grave danno lo stop alla ricerca sugli embrioni»

...ICA 15 MAGGIO 2005 - N. 132 — PAGINA 35

APPELLO DI 77 NOBEL

Sì alla ricerca sulle staminali embrionali

A I 77 Nobel che hanno firmato l'appello all'Onu pubblicato qui in esclusiva

Derivation of midbrain dopamine neurons from human embryonic stem cells

PNAS,2004

STAMINALI EMBRIONALI E PARKINSON

Derivation of midbrain dopamine neurons from human embryonic stem cells

Anselme L. Perrier*, Viviane Tabar*, Tiziano Barberi*, Maria E. Rubio†, Juan Bruses‡, Norbert Topf§, Neil L. Harrison§, and Lorenz Studer*¶

PNAS 101:12543, 2004

nature medicine

ARTICLES

Ottobre 2006

Functional engraftment of human ES cell–derived dopaminergic neurons enriched by coculture with telomerase-immortalized midbrain astrocytes

Neeta S Roy[1], Carine Cleren[1], Shashi K Singh[1], Lichuan Yang[1], M Flint Beal[1] & Steven A Goldman[1,2]

NEWS

Ottobre 2006

Published online: 22 October 2006; |
doi:10.1038/news061016-16

Stem-cell treatment for Parkinson's brings mixed results

Almost total relief of symptoms tempered by hints of cancerous side effects.

18000 NEUROLOGI AMERICANI CHIEDONO DI STUDIARE LE STAMINALI EMBRIONALI

Special Article

Position statement regarding the use of embryonic and adult human stem cells in biomedical research

American Academy of Neurology and American Neurological Association

Preamble. The American Academy of Neurology (AAN) and the American Neurological Association (ANA), organizations representing over 18,000 neurologists and neuroscience professionals, support government funding of basic, clinical, and translational research that will ultimately benefit patients with neurologic diseases. The AAN and ANA believe that the use of human pluripotent stem cells (also known as human embryonic stem cells) in biomedical research may have enormous potential to benefit people affected by neurologic disease throughout the world. In particular, the research involving such cells could improve the lives of many Americans suffering from neurologic diseases, examples of which are ALS (Lou Gehrig disease), Alzheimer disease, epilepsy, Huntington disease, multiple sclerosis, Parkinson disease, spinal cord injury, and stroke.

While the potential of embryonic stem cell research to result in breakthrough therapies is real, it is important to recognize that the translation of re-

Bioethics January 2004 report, Monitoring Stem Cell Research, "This research is expensive and technically challenging, and requires scientists willing to take a long perspective in order to discover, through painstaking research, which combinations of techniques could turn out to be successful. Strong financial support, public and private, will be indispensable to achieving success."[1]

All research, including stem cell research, must meet the standards of scientific and ethical oversight by external peer review. The AAN and ANA promote the highest standards for oversight, which many consider to be that attached to federally funded research. In 2000, the NIH issued *Guidelines for Research Involving Human Pluripotent Stem Cells*, enabling scientists to conduct federally-funded embryonic stem cell research (ESCR) within the constraints of federal oversight and standards.[2] Those guidelines were altered by Presidential order on August 9, 2001, limiting ESCR to stem cell lines that

IL PLAUSO DELL'ISSCR ALL'INIZIATIVA DI MUSSI

SAVE THE DATE!
ISSCR 4th Annual Meeting
June 29 – July 1, 2006
Metro Toronto Convention Centre
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Open letter to President Romano Prodi and Ministers of the Italian Republic

From: Gordon Keller, President, International Society for Stem Cell Research
Co-signatory: Austin Smith, Coordinator, European Consortium for Stem Cell Research

The International Society for Stem Cell Research (ISSCR) has become aware of the recent decision of the Italian Minister of Research and University, Fabio Mussi, to withdraw Italy's signature from an 'Ethical Declaration against Human Embryonic Stem Cell Research', which was placed by the previous government in the European Union (*).

While having no direct impact on the current Italian legislation (**), this decision removes a significant barrier to the freedom of scientific research and medical advancement in the European Union.

We as a society, endorse Minister Mussi and the stance of the new Government of Italy on this issue. The withdrawal from the Ethical Declaration is consistent with the opinion of the European Group on Ethics (***) and is of great importance for citizens in those European countries that have come to democratic decisions that research on human embryonic stem cells is necessary, legitimate and ethical.

Europe has made major historical contributions in the field of fundamental stem cell research and is well-positioned to translate this knowledge to the clinic and develop future treatments for human disease. Italy is no longer blocking scientific progress for universal benefit. We applaud this honourable decision that takes into full consideration pluralism of ideas and principles. On the other hand, reversal of the decision made by the Minister would have a negative effect on the whole European and International scientific community, slowing research progress towards regenerative therapies.

nature Vol 447|7 June 2007

NEWS

Simple switch turns cells embryonic

Research reported this week by three different groups shows that normal skin cells can be reprogrammed to an embryonic state in mice[1–3]. The race is now on to apply the surprisingly straightforward procedure to human cells.

If researchers succeed, it will make it relatively easy to produce cells that seem indistinguishable from embryonic stem cells, and that are genetically matched to individual patients. There are limits to how useful and safe these would be for therapeutic use in the near term, but they should quickly prove a boon in the lab.

"It would change the way we see things quite dramatically," says Alan Trounson of Monash University in Victoria, Australia. Trounson wasn't involved in the new work but says he plans to start using the technique "tomorrow". "I can think of a dozen experiments right now — and they're all good ones," he says.

"It's unbelievable, just amazing. It's like Dolly. It's that type of accomplishment."

an adult cell and then forcing the cell to divide to create an early-stage embryo, from which the stem cells can be harvested. Those barriers may have now been broken down.

"Neither eggs nor embryos are necessary. I've never worked with either," says Shinya Yamanaka of Kyoto University, who has pioneered the new technique.

Last year, Yamanaka introduced a system that uses mouse fibroblasts, a common cell type that can easily be harvested from skin, instead of eggs[4]. Four genes, which code for four specific proteins known as transcription factors, are transferred into the cells using retroviruses. The proteins trigger the expression of other genes that lead the cells to become pluripotent, meaning that they could potentially become any of the body's cells. Yamanaka calls them induced pluripotent stem cells (iPS cells). "It's easy.

was not comfortable with the term 'pluripotent' last year," says Hans Schöler, a stem-cell specialist at the Max Planck Institute for Molecular Biomedicine in Münster who is not involved with any of the three articles.

This week, Yamanaka presents a second generation of iPS cells[1], which pass all these tests. In addition, a group led by Rudolf Jaenisch[2] at the Whitehead Institute for Biomedical Research in Cambridge, Massachusetts, and a collaborative effort[3] between Konrad Hochedlinger of the Harvard Stem Cell Institute and Kathrin Plath of the University of California, Los Angeles, used the same four factors and got strikingly similar results.

"It's a relief as some people questioned our results, especially after the Hwang scandal," says Yamanaka, referring to the irreproducible cloning work of Woo Suk Hwang, which turned out to be fraudulent. Schöler agrees: "Now we can be confident that this is some-

NATURE|Vol 448|19 July 2007

STEM CELLS

The magic brew

Janet Rossant

Researchers have engineered embryonic stem-like cells from normal mouse skin cells. If this method can be translated to humans, patient-specific stem cells could be made without the use of donated eggs or embryos.

iPSCs = induced Pluripotent Stem Cells

Induction of Pluripotent Stem Cells from Mouse Embryonic and Adult Fibroblast Cultures by Defined Factors

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DOI 10.1016/j.cell.2006.07.024

SUMMARY

Differentiated cells can be reprogrammed to an embryonic-like state by transfer of nuclear contents into oocytes or by fusion with embryonic stem (ES) cells. Little is known about factors that induce this reprogramming. Here, we demonstrate induction of pluripotent stem cells from mouse embryonic or adult fibroblasts by introducing four factors, Oct3/4, Sox2, c-Myc, and Klf4, under ES cell culture conditions. Unexpectedly, Nanog was dispensable. These cells, which we designated iPS (induced pluripotent stem) cells, exhibit the morphology and growth properties of ES cells and express ES cell marker genes. Subcutaneous transplantation of iPS cells into nude mice resulted in tumors containing a variety of tissues from all three germ layers. Following injection into blastocysts, iPS cells contributed to mouse embryonic development. These data demonstrate that pluripotent stem cells can be directly generated from fibroblast cultures by the addition of only a few defined factors.

or by fusion with ES cells (Cowan et al., 2005; Tada et al., 2001), indicating that unfertilized eggs and ES cells contain factors that can confer totipotency or pluripotency to somatic cells. We hypothesized that the factors that play important roles in the maintenance of ES cell identity also play pivotal roles in the induction of pluripotency in somatic cells.

Several transcription factors, including Oct3/4 (Nichols et al., 1998; Niwa et al., 2000), Sox2 (Avilion et al., 2003), and Nanog (Chambers et al., 2003; Mitsui et al., 2003), function in the maintenance of pluripotency in both early embryos and ES cells. Several genes that are frequently upregulated in tumors, such as *Stat3* (Matsuda et al., 1999; Niwa et al., 1998), *E-Ras* (Takahashi et al., 2003), *c-myc* (Cartwright et al., 2005), *Klf4* (Li et al., 2005), and *β-catenin* (Kielman et al., 2002; Sato et al., 2004), have been shown to contribute to the long-term maintenance of the ES cell phenotype and the rapid proliferation of ES cells in culture. In addition, we have identified several other genes that are specifically expressed in ES cells (Maruyama et al., 2005; Mitsui et al., 2003).

In this study, we examined whether these factors could induce pluripotency in somatic cells. By combining four selected factors, we were able to generate pluripotent cells, which we call induced pluripotent stem (iPS) cells, directly from mouse embryonic or adult fibroblast cultures.

Induction of Pluripotent Stem Cells from Adult Human Fibroblasts by Defined Factors

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DOI 10.1016/j.cell.2007.11.019

SUMMARY

Successful reprogramming of differentiated human somatic cells into a pluripotent state would allow creation of patient- and disease-specific stem cells. We previously reported generation of induced pluripotent stem (iPS) cells, capable of germline transmission, from mouse somatic cells by transduction of four defined transcription factors. Here, we demonstrate the generation of iPS cells from adult human dermal fibroblasts with the same four factors: Oct3/4, Sox2, Klf4, and c-Myc. Human iPS cells were similar to human embryonic stem (ES) cells in morphology, proliferation, surface antigens, gene expression, epigenetic status of pluripotent cell-specific genes, and telomerase activity. Furthermore, these cells could differentiate into cell types of the three germ layers in vitro and in teratomas. These findings demonstrate that iPS cells can be generated from adult human fibroblasts.

issues is to induce pluripotent status in somatic cells by direct reprogramming (Yamanaka, 2007).

We showed that induced pluripotent stem (iPS) cells can be generated from mouse embryonic fibroblasts (MEF) and adult mouse tail-tip fibroblasts by the retrovirus-mediated transfection of four transcription factors, namely Oct3/4, Sox2, c-Myc, and Klf4 (Takahashi and Yamanaka, 2006). Mouse iPS cells are indistinguishable from ES cells in morphology, proliferation, gene expression, and teratoma formation. Furthermore, when transplanted into blastocysts, mouse iPS cells can give rise to adult chimeras, which are competent for germline transmission (Maherali et al., 2007; Okita et al., 2007; Wernig et al., 2007). These results are proof of principle that pluripotent stem cells can be generated from somatic cells by the combination of a small number of factors.

In the current study, we sought to generate iPS cells from adult human somatic cells by optimizing retroviral transduction in human fibroblasts and subsequent culture conditions. These efforts have enabled us to generate iPS cells from adult human dermal fibroblasts and other human somatic cells, which are comparable to human ES cells in their differentiation potential in vitro and in teratomas.

Sciencexpress Report

Induced Pluripotent Stem Cell Lines Derived from Human Somatic Cells

Junying Yu,[1,2]* Maxim A. Vodyanik,[2] Kim Smuga-Otto,[1,2] Jessica Antosiewicz-Bourget,[1,2] Jennifer L. Frane,[1] Shulan Tian,[3] Jeff Nie,[3] Gudrun A. Jonsdottir,[3] Victor Ruotti,[3] Ron Stewart,[3] Igor I. Slukvin,[2,4] James A. Thomson[1,2,5]*

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Somatic cell nuclear transfer allows trans-acting factors present in the mammalian oocyte to reprogram somatic cell nuclei to an undifferentiated state. Here we show that four factors (*OCT4*, *SOX2*, *NANOG*, and *LIN28*) are sufficient to reprogram human somatic cells to pluripotent stem cells that exhibit the essential characteristics of embryonic stem cells. These human induced pluripotent stem cells have normal karyotypes, express telomerase activity, express cell surface markers and genes that characterize human ES cells, and maintain the developmental potential to differentiate into advanced derivatives of all three primary germ layers. Such human induced pluripotent cell lines should be useful in the production of new disease models and in drug development as well as application in transplantation medicine once technical limitations (for example, mutation through viral integration) are eliminated.

demonstrate that *OCT4*, *SOX2*, *NANOG*, and *LIN28* are sufficient to reprogram human somatic cells.

Human ES cells can reprogram myeloid precursors through cell fusion (*7*). To identify candidate reprogramming factors, we compiled a list of genes with enriched expression in human ES cells relative to myeloid precursors, and prioritized the list based on known involvement in the establishment or maintenance of pluripotency (table S1). We then cloned these genes into a lentiviral vector (fig. S1) to screen for combinations of genes that could reprogram the differentiated derivatives of an *OCT4* knock-in human ES cell line generated through homologous recombination (*8*). In this cell line, the expression of neomycin phosphotransferase, which make cells resistant to geneticin, is driven by an endogenous *OCT4* promoter, a gene that is highly expressed in pluripotent cells but not in differentiated cells. Thus reprogramming events reactivating the *OCT4* promoter can

21.11.07

nature Vol 448|19 July 2007|doi:10.1038/nature05944

ARTICLES

In vitro reprogramming of fibroblasts into a pluripotent ES-cell-like state

Marius Wernig[1]*, Alexander Meissner[1]*, Ruth Foreman[1,2]*, Tobias Brambrink[1]*, Manching Ku[3]*, Konrad Hochedlinger[1]†, Bradley E. Bernstein[3,4,5] & Rudolf Jaenisch[1,2]

Nuclear transplantation can reprogramme a somatic genome back into an embryonic epigenetic state, and the reprogrammed nucleus can create a cloned animal or produce pluripotent embryonic stem cells. One potential use of the nuclear cloning approach is the derivation of 'customized' embryonic stem (ES) cells for patient-specific cell treatment, but technical and ethical considerations impede the therapeutic application of this technology. Reprogramming of fibroblasts to a pluripotent state can be induced *in vitro* through ectopic expression of the four transcription factors Oct4 (also called Oct3/4 or Pou5f1), Sox2, c-Myc and Klf4. Here we show that DNA methylation, gene expression and chromatin state of such induced reprogrammed stem cells are similar to those of ES cells. Notably, the cells—derived from mouse fibroblasts—can form viable chimaeras, can contribute to the germ line and can generate live late-term embryos when injected into tetraploid blastocysts. Our results show that the biological potency and epigenetic state of *in-vitro*-reprogrammed induced pluripotent stem cells are indistinguishable from those of ES cells.
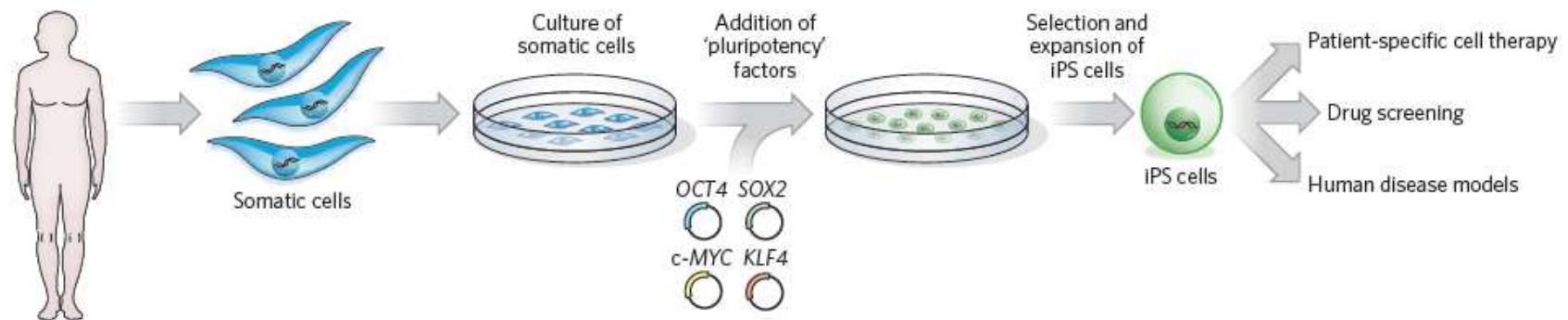
Figure 4 | Applications of iPS cells. To generate iPS cells, fibroblasts (or another type of adult somatic cell) are transduced with retroviruses encoding four pluripotency factors (SOX2, KLF4, c-MYC and OCT4)[56,63]. Fully reprogrammed iPS cells have similar properties to ES cells. They are competent to form teratomas on injection into mice and are capable of generating progeny. A patient's cells can be used to derive iPS cells, which can then be induced to undergo differentiation into various types of somatic cell, all with the same genetic information as the patient. For example, dopaminergic neurons could be generated from the cells of a patient with Parkinson's disease and then transplanted to replace those neurons that have been lost. These differentiated cells can also be used in disease models for studying the molecular basis of a broad range of human diseases that are otherwise difficult to study (for instance, those that affect brain cells) and for screening the efficacy and safety of drug candidates for treating these diseases.

Il ricercatore James Thomson che per primo ha prodotto staminali embrionali umane nel 1998

Il ricercatore Shinya Yamanaka riceve a Hong Kong il premio della Fondazione Shaw

Darwin , 31, maggio/giugno 2009

LA STAMPA
MERCOLEDÌ 21 NOVEMBRE 2007 | TuttoScienze | III

Staminali sì, ma senza embrioni

Genetica. Trasferendo quattro geni, le cellule della pelle ritornano bambine: "Così si risolvono i problemi etici"
Ricerca americana e giapponese: "Non immaginavamo che potesse essere tanto semplice. Si apre una nuova era"

MAUR

21.11.07
Repubblica

ad aggiungere un minor numero di geni, senza risultati

CELLULE NORMALI

provenienti da parti diverse del corpo umano, isolate e fatte crescere in coltura

6 Pancreas
7 Cuore

Fonte: Università di Kyoto

La rivoluzione delle staminali così le cellule "ringiovaniscono"

Esperimenti senza embrioni. Bush: bene, non si distrugge la vita

MARIO REGGIO

ROMA — Cellule adulte di pelle umana "riprogrammate" inserendo nel Dna pochissimi geni che, come per incanto, tornano "bambine". Senza ricorrere alla clonazione e senza distruggere embrioni. La sperimentazione è stata conclusa da due équipe di ricercatori, uno statunitense e l'altro giapponese, che hanno lavorato in modo indipendente ed impiegato tecniche diverse anche se simili. I risultati sono stati pubblicati rispettivamente sulle edizioni online di "Science" e "Cell". Per comprendere la portata dei risultati delle ricerche basta registrare la presa di posizione del presidente degli Stati Uniti: «La Casa Bianca accoglie con grande favore i risultati dello studio innovati-

bilire che i due tipi di cellule possono essere equivalenti in un futuro uso terapeutico. «Le cellule create in laboratorio fanno esattamente ciò che le staminali embrionali sono capaci di fare», ha osservato Thomson. «Forse — ha aggiunto — sono clinicamente ancora più rilevanti di quelle embrionali, perchè non dovrebbero dare problemi di rigetto». Ottimi-

sta, Yamanaka, sul futuro della ricerca: «ora dovremmo essere capaci di generare cellule staminali umane e ottenere vari tipi di cellule, ad esempio cardiache, epatiche, neurali. Queste saranno estremamente utili per studiare le malattie, testare farmaci e, in futuro, aprire la via a terapie cellulari su misura». Il mondo scientifico italiano plaude ai risultati delle ricerche. «Sono risultati che nascono da una ricerca molto solida», commenta Elena Cattaneo, direttrice del Laboratorio cellule staminali dell'Università di Milano. «La ricerca sulle staminali embrionali resta indispensabile», afferma Giuseppe Novelli, docente di Genetica a Tor Vergata, «senza di loro non sarebbero stati raggiunti questi risultati».

21.11.07

iPS derivate da pazienti (studio meccanismi d'azione, nuovi farmaci…)

Vol 457|15 January 2009 nature

NEWS & VIEWS

STEM CELLS

Tailor-made diseased neurons

Michael Sendtner

How can we investigate a disease affecting neurons, which cannot be isolated from patients for analysis? As the study of one neurological disord

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ARTICLES

Induced pluripotent stem cells from a spinal muscular atrophy patient

Allison D. Ebert[1,2], Junying Yu[3], Ferrill F. Rose Jr[4], Virginia B. Mattis[4], Christian L. Lorson[4], James A. Thomson[2,3,5] & Clive N. Svendsen[1,2,5,6]

Spinal muscular atrophy is one of the most common inherited forms of neurological disease leading to infant mortality. Patients have selective loss of lower motor neurons resulting in muscle weakness, paralysis and often death. Although patient fibroblasts have been used extensively to study spinal muscular atrophy, motor neurons have a unique anatomy and physiology which may underlie their vulnerability to the disease process. Here we report the generation of induced pluripotent stem cells from skin fibroblast samples taken from a child with spinal muscular atrophy. These cells expanded robustly in culture, maintained the disease genotype and generated motor neurons that showed selective deficits compared to those derived from the child's unaffected mother. This is the first study to show that human induced pluripotent stem cells can be used to model the specific pathology seen in a genetically inherited disease. As such, it represents a promising resource to study disease mechanisms, screen new drug compounds and develop new therapies.

Resource

Cell

Disease-Specific Induced Pluripotent Stem Cells

In-Hyun Park,[1,7] Natasha Arora,[1,7] Hongguang Huo,[1,7] Nimet Maherali,[2,3,7] Tim Ahfeldt,[2,5,7] Akiko Shimamura,[4] M. William Lensch,[1,7,9] Chad Cowan,[2,6,7] Konrad Hochedlinger,[2,7] and George Q. Daley[1,7,8,9,*]

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SUMMARY

Tissue culture of immortal cell strains from diseased patients is an invaluable resource for medical research but is largely limited to tumor cell lines or transformed derivatives of native tissues. Here we describe the generation of induced pluripotent stem (iPS) cells from patients with a variety of genetic diseases with either Mendelian or complex inheritance; these diseases include adenosine deaminase deficiency-related severe combined immunodeficiency (ADA-SCID), Shwachman-Bodian-Diamond syndrome (SBDS), Gaucher disease (GD) type III, Duchenne (DMD) and Becker muscular dystrophy (BMD), Parkinson disease (PD), Huntington disease (HD), juvenile-onset, type 1 diabetes mellitus (JDM), Down syndrome (DS)/trisomy 21, and the carrier state of Lesch-Nyhan syndrome. Such disease-specific stem cells offer an unprecedented opportunity to recapitulate both normal and pathologic human tissue formation in vitro, thereby enabling disease investigation and drug development.

tissues or are genetically modified to drive immortal growth (Grimm, 2004). Primary human cells have a limited life span in culture, a constraint that thwarts inquiry into the regulation of tissue formation, regeneration, and repair. Indeed, many human cell types have never faithfully been adapted for growth in vitro, and the lack of accessible models of normal and pathologic tissue formation has rendered many important questions in human development and disease pathogenesis inaccessible.

Human embryonic stem cells isolated from excess embryos from in vitro fertilization clinics represent an immortal propagation of pluripotent cells that theoretically can generate any cell type within the human body (Lerou et al., 2008; Murry and Keller, 2008). Human embryonic stem cells allow investigators to explore early human development through in vitro differentiation, which recapitulates aspects of normal gastrulation and tissue formation. Embryos shown to carry genetic diseases by virtue of preimplantation genetic diagnosis (PGD; genetic analysis of single blastomeres obtained by embryo biopsy) can yield stem cell lines that model single-gene disorders (Verlinsky et al., 2005), but the vast majority of diseases that show more complex genetic patterns of inheritance are not represented in this pool.

A tractable method for establishing immortal cultures of pluripotent stem cells from diseased individuals would not only facilitate disease research but also lay a foundation for producing

Resource

Parkinson's Disease Patient-Derived Induced Pluripotent Stem Cells Free of Viral Reprogramming Factors

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DOI 10.1016/j.cell.2009.02.013

SUMMARY

Induced pluripotent stem cells (iPSCs) derived from somatic cells of patients represent a powerful tool for biomedical research and may provide a source for replacement therapies. However, the use of viruses encoding the reprogramming factors represents a major limitation of the current technology since even low vector expression may alter the differentiation potential of the iPSCs or induce malignant transformation. Here, we show that fibroblasts from five patients with idiopathic Parkinson's disease can be efficiently reprogrammed and subsequently differentiated into dopaminergic neurons. Moreover, we derived hiPSCs free of reprogramming factors using Cre-recombinase excisable viruses. Factor-free hiPSCs maintain a pluripotent state and show a global gene expression profile, more closely related to hESCs than to hiPSCs carrying the transgenes. Our results indicate that residual transgene expression in virus-carrying hiPSCs can affect their molecular characteristics and that factor-free hiPSCs therefore represent a more suitable source of cells for modeling of human disease.

et al., 2008; Ebert et al., 2009; Park et al., 2008a). hiPSCs, characterized by their ability to self-renew and to differentiate into any cell type of the body, are predicted to become a powerful tool for biomedical research as well as a source for cell-replacement therapies. Although the realization of ESC/induced pluripotent stem cell (iPSC)-based therapies is still at an early stage of development, the possibility of modeling human disease in vitro could make patient-specific hiPSCs immediately valuable. This is particularly relevant for diseases of the central nervous system (CNS) such as Parkinson's disease (PD), where primary neuronal tissue is not available.

PD is the second most common chronic progressive neurodegenerative disorder and is characterized primarily by major loss of nigrostriatal dopaminergic neurons. The discovery of genes linked to rare familial forms of PD has provided vital clues in understanding the cellular and molecular pathogenesis of the disease (Gasser, 2007; Schulz, 2008). However, the majority of cases are sporadic, not linked to a known genetic mutation, and likely the result of complex interactions between genetic and environmental factors (de Lau and Breteler, 2006). One of the major reasons for the lack of understanding of the underlying pathophysiology of PD is the paucity of reliable experimental models that recapitulate all features of the human disease. The derivation of PD patient-specific hiPSCs and subsequent differentiation into dopaminergic neurons would provide patient-specific in vitro models that are otherwise experimentally not accessible.

ALZHEIMER'S DISEASE

Stem Cells to Neurons

Scientists create neurons whose early death causes memory loss

QUOTE

“If this isn't more motivation for people to maintain some degree of

March 3, 2011 | Research

From Stem Cells to Neurons Lost in Alzheimer's

Scientists crack code to create neurons whose early death causes memory loss

By Marla Paul

CHICAGO --- Northwestern Medicine researchers for the first time have transformed a human embryonic stem cell into a critical type of neuron that dies early in Alzheimer's disease and is a major cause of memory loss.

This new ability to reprogram stem cells and grow a limitless supply of the human neurons will enable a rapid wave of drug testing for Alzheimer's disease, allow researchers to study why the neurons die and could potentially lead to transplanting the new neurons into people with Alzheimer's.

The paper will be published March 4 in the journal Stem Cells.

These critical neurons, called basal forebrain cholinergic neurons, help the hippocampus retrieve memories in the brain. In early Alzheimer's, the ability to retrieve memories is lost, not the memories themselves. There is a relatively small population of

JHU team creates stem cells from schizophrenia patients

Mi piace · Piace a 2 persone. Registrazione per vedere cosa piace ai tuoi amici. Share this story

March 21, 2011
By Maryalice Yakutchik
Johns Hopkins Medicine

Filed under Around Hopkins

Using skin cells from adult siblings with schizophrenia and a genetic mutation linked to major mental illnesses, Johns Hopkins researchers have created induced pluripotent stem cells using a new and improved “clean” technique.

Reporting online Feb. 22 in *Molecular Psychiatry*, the team confirms the establishment of two new lines ; cells with mutations in the gene named Disrupted In Schizophrenia 1, or DISC1. They made the ısing a nonviral “epiosomal vector” that jump-starts the reprogramming machinery of cells without ying their original genetic content with foreign DNA from a virus.

tem cells from these two new lines, the scientists say, can be coaxed to become brain cells such ırons. Because they have the DISC1 mutation, they stand to play an important role in the ning of drugs for treatments of major mental illnesses such as schizophrenia, bipolar disorder and depression, as well as provide clues about the causes of these diseases.

t people think of stem cells only as potential transplant therapy to replace damaged cells or tissue, ɪr psychiatric diseases, which have proven a challenge to scientific understanding just as a sheer :hallenges a climber, these cells provide a toehold,” said Russell L. Margolis, a professor of iatry and neurology and director of the Johns Hopkins Schizophrenia Program. “Nature put in ı few little grab holds, and now we are generating our own so we can scale the cliff of mental ; faster.”

Athersys Announces Initiation of Patient Enrollment for Phase II Clinical Trial in Inflammatory Bowel Disease

Athersys and Pfizer Collaborate on Proprietary Stem Cell Therapy for Ulcerative Colitis

CLEVELAND, March 14, 2011 (GLOBE NEWSWIRE) -- Athersys, Inc. (Nasdaq:ATHX) announced today the initiation of patient enrollment, and dosing of the first patient for a Phase II clinical trial evaluating the safety and efficacy of administration of MultiStem®, Athersys' allogeneic cell therapy product for the treatment of ulcerative colitis (UC). This Phase II clinical trial is part of a strategic global collaboration between Athersys and Pfizer Inc. (NYSE:PFE) to investigate MultiStem for the treatment of inflammatory bowel disease (IBD).

The Phase II study is a randomized, double-blind, placebo-controlled, multi-center study designed to investigate the safety and efficacy of MultiStem in subjects with moderate to severe UC. The trial will be conducted at multiple clinical sites in North America and Europe, and is expected to include up to approximately 128 patients. Individuals participating in the study will receive multiple doses of either MultiStem or placebo, administered over a period of several weeks. Primary safety and efficacy endpoints will include endoscopic evaluation at baseline and at eight weeks, with a follow-up of all patients through twelve months.

About MultiStem

MultiStem is a patented and proprietary product candidate that can be manufactured on a large scale, subsequently frozen and later thawed and administered, similar to traditional biologics. MultiStem consists of a clinical grade preparation of non-embryonic stem cells obtained from bone marrow that have the potential to produce a range of factors and form multiple cell types. MultiStem appears to work through several mechanisms, but a primary mechanism appears to be the production of therapeutic proteins and other molecules produced in response to inflammation and tissue damage. Athersys believes that MultiStem may represent a unique "off-the-shelf" stem cell product based on its apparent ability to be used without tissue matching or immunosuppression and its capacity for large scale production.

any individuals,
Executive
commencing

NATURE|Vol 465|10 June 2010|doi:10.1038/nature09229

Nuclear reprogramming to a pluripotent state by three approaches

Shinya Yamanaka[1,2] & Helen M. Blau[3]

The stable states of differentiated cells are now known to be controlled by dynamic mechanisms that can easily be perturbed. An adult cell can therefore be reprogrammed, altering its pattern of gene expression, and hence its fate, to that typical of another cell type. This has been shown by three distinct experimental approaches to nuclear reprogramming: nuclear transfer, cell fusion and transcription-factor transduction. Using these approaches, nuclei from 'terminally differentiated' somatic cells can be induced to express genes that are typical of embryonic stem cells, which can differentiate to form all of the cell types in the body. This remarkable discovery of cellular plasticity has important medical applications.

nature International weekly journal of science

Editor's Summary

3 September 2009

Mice from iPS cells

Since iPS (induced pluripotent stem) cells arrived on the scene in 2006, their properties have been measured against the yardstick of the true embryonic stem cells that they mimic. A clutch of recent papers, two of them published in this issue, reports the production of viable adult mice from iPS cells, a notable technical feat that shows that these cells are very close indeed to embryonic cells in their potential to produce cells for all tissues and all organs. Zhao *et al*. used a technique called tetraploid complementation, in which chimaeric mice are generated from injected pluripotent cells, and the embryonic tissue is derived solely from the injected cells. Boland *et al*. produced fertile adult mice derived entirely from iPS cells generated by inducible genetic reprogramming of mouse embryonic fibroblasts. The availability of these mice will provide a new resource for the study of iPS cell-derived tissues for both research and cell replacement therapy applications.

Vol 461|3 September 2009|doi:10.1038/nature08310

LETTERS

Adult mice generated from induced pluripotent stem cells

Michael J. Boland[1]*, Jennifer L. Hazen[1]*, Kristopher L. Nazor[1]*, Alberto R. Rodriguez[2], Wesley Gifford[3], Greg Martin[2], Sergey Kupriyanov[2] & Kristin K. Baldwin[1]

Recent landmark experiments have shown that transient over-expression of a small number of transcription factors can re-program differentiated cells into induced pluripotent stem (iPS) cells that resemble embryonic stem (ES) cells[1–7]. These iPS cells hold great promise for medicine because they have the potential to generate patient-specific cell types for cell replacement therapy and produce *in vitro* models of disease, without requiring embry-onic tissues or oocytes[8–10]. Although current iPS cell lines resemble ES cells, they have not passed the most stringent test of pluripotency by generating full-term or adult mice in tetraploid complementa-tion assays[3,11], raising questions as to whether they are sufficiently potent to generate all of the cell types in an organism. Whether this difference between iPS and ES cells reflects intrinsic limitations of direct reprogramming is not known. Here we report fertile adult mice derived entirely from iPS cells that we generated by inducible genetic reprogramming of mouse embryonic fibroblasts. Producing adult mice derived entirely from a reprogrammed fibroblast shows that all features of a differentiated cell can be restored to an embry-onic level of pluripotency without exposure to unknown ooplasmic factors. Comparing these fully pluripotent iPS cell lines to less developmentally potent lines may reveal molecular markers of different pluripotent states. Furthermore, mice derived entirely from iPS cells will provide a new resource to assess the functional and genomic stability of cells and tissues derived from iPS cells, which is important to validate their utility in cell replacement therapy and research applications.

marking strategy to distinguish between host blastocyst and iPS-derived cells. We established mouse embryonic fibroblasts (MEFs) from animals generated by a cross of two mouse lines (*Pcdh21*/Cre and Z/EG, Fig. 1a). The Z/EG transgene labels most cells in an animal with a visible marker (β-geo, a fusion of the β-galactosidase and neomycin genes)[17], whereas the *Pcdh21*/Cre modification results in Cre expression in rare neuronal subtypes, but not in ES cells[18]. Cre expression causes excision of the floxed β-geo gene, resulting in green fluorescent protein (GFP) expression in olfactory bulb mitral cells, a feature we exploit later (Fig. 1a).

We reasoned that the inappropriate expression of reprogramming genes during development could inhibit embryonic and postnatal development. Therefore, we designed a drug-inducible lentiviral reprogramming strategy to achieve tight control of transgene expres-sion in iPS cells and their derivatives (Fig. 1b)[19]. The four original reprogramming factors (*Oct4* (also known as *Pou5f1*), *Sox2*, *Klf4* and *c-Myc*) were placed under control of the tetO promoter, which is activated by the reverse tetracycline transactivator (rtTA) protein in the presence of the tetracycline analogue doxycycline (dox). We used an enhanced version of the rtTA transcriptional activator protein (rtTAM2.2) that induces higher gene expression levels than the rtTAM2 protein[20]. To promote complete reprogramming and to facil-itate isolation of fully reprogrammed iPS cells we exposed MEFs to the histone deacetylase inhibitor valproic acid (VPA), which has been reported to enhance reprogramming efficiency and to select against incompletely reprogrammed cells by inhibiting cell division[21,22] (see

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News

There will be blood

Direct conversion of cell types could offer safer, simpler treatments than stem cells.

Ewen Callaway

In a feat of cellular alchemy, human skin cells have been transformed into blood cells without first being sent through a primordial, stem-cell-like state. For the developers of patient-specific cell therapies, the result could be safer and simpler than induced pluripotent stem (iPS) cells — reprogrammed adult cells that can differentiate into many cell types.

Published in *Nature*[1], the study follows work earlier this year showing that fibroblast cells from mouse skin can be transformed into neurons[2] and heart muscle[3]. However, it is the first study to accomplish direct reprogramming with human cells, and the first to create progenitor cells — in this case for blood. "It takes us a step along the line to believing that you can produce anything from almost anything," says Ian Wilmut, director of the Medical Research Council Centre for Regenerative Medicine in Edinburgh, UK, who was not involved in the study.

Mickie Bhatia, a stem-cell researcher at McMaster University in Hamilton, Canada, and his colleagues infected skin cells with a virus that inserted the *OCT4* gene, then they grew the cells in a soup of immune-system stimulating proteins called cytokines. The gene's product, the OCT4 protein, is one of a handful of factors used to transform fibroblasts into iPS cells, but Bhatia's team found no evidence that the blood progenitor cells they made went through an embryonic state. For instance, the cells did not cause mice to develop teratomas — tumours that are characteristic of pluripotent

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ARTICLE

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Direct conversion of human fibroblasts to multilineage blood progenitors

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As is the case for embryo-derived stem cells, application of reprogrammed human induced pluripotent stem cells is limited by our understanding of lineage specification. Here we demonstrate the ability to generate progenitors and mature cells of the haematopoietic fate directly from human dermal fibroblasts without establishing pluripotency. Ectopic expression of OCT4 (also called POU5F1)-activated haematopoietic transcription factors, together with specific cytokine treatment, allowed generation of cells expressing the pan-leukocyte marker CD45. These unique fibroblast-derived cells gave rise to granulocytic, monocytic, megakaryocytic and erythroid lineages, and demonstrated *in vivo* engraftment capacity. We note that adult haematopoietic programs are activated, consistent with bypassing the pluripotent state to generate blood fate: this is distinct from haematopoiesis involving pluripotent stem cells, where embryonic programs are activated. These findings demonstrate restoration of multipotency from human fibroblasts, and suggest an alternative approach to cellular reprogramming for autologous cell-replacement therapies that avoids complications associated with the use of human pluripotent stem cells.

Mechanisms that govern induced pluripotent stem cell (iPSC) reprogramming from human fibroblasts remain poorly understood[1]. The process is further complicated by cellular intermediates that fail to establish a stable pluripotent state, potentially due to the inability to establish the ideal expression context of reprogramming factors to complete pluripotency induction[2–5]. These intermediates co-express genes associated with several differentiated lineages (neurons, epidermis and mesoderm)[4,5], raising the possibility that under unique conditions, fibroblasts could be induced to differentiate towards specified lineages. This maybe akin to recent demonstrations where fibroblasts were converted into single cell types, such as neurons, cardiomyocytes and macrophage-like cells[6–8]. While these studies have examined fibroblast conversion in the murine model, a similar process remains to be extrapolated towards human applications.

Our preliminary observations indicated that human dermal fibroblasts (Fibs) predominantly expressing OCT4 during the pluripotent reprogramming process express lineage differentiation markers that include the human pan-haematopoietic marker CD45. While both OCT2 (also called POU2F2) and OCT1 (also called POU2F1) bind similar DNA target motifs to OCT4 (ref. 9), and play a role in $CD45^+$ cells preferentially express OCT4 while demonstrating low levels of SOX2 and NANOG (Supplementary Fig. 2d, e). This suggested that Fib-derived intermediates could acquire a distinct lineage phenotype.

On the basis of these results, we compared the role of OCT4 during colony emergence from two sources of Fibs (adult dermal and neonatal foreskin) with that of NANOG or SOX2 alone (Fig. 1a). Transduced versus untransduced Fibs were examined between 14 and 21 days post-transduction (D14–D21; Supplementary Fig. 3). Unlike untransduced Fibs, or Fibs transduced with SOX2 ($Fibs^{SOX2}$) or NANOG ($Fibs^{NANOG}$), Fibs expressing OCT4 (Fib^{OCT4}) gave rise to colonies (Fig. 1a, Supplementary Fig. 3b) and exhibited OCT4 expression at levels similar to those detected in established iPSCs (Fig. 1b). $Fibs^{OCT4}$ exclusively gave rise to haematopoietic-like $CD45^+$ cells (Fig. 1c). Furthermore, $CD45^+$ cells ($CD45^+Fibs^{OCT4}$) showed an increase in OCT4 expression (Supplementary Fig. 3c) with a concomitant decrease in the fibroblast specific gene expression[15] (Fig. 1d). Approximately 1,000 genes were downregulated and an equal number upregulated at D4, resulting in a shift towards the $FibCD45^+$ phenotype (Supplementary Table 1). To characterize and enhance emergence of $CD45^+$ Fibs, we used Flt3

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ARTICLES

Direct conversion of fibroblasts to functional neurons by defined factors

Thomas Vierbuchen[1,2], Austin Ostermeier[1,2], Zhiping P. Pang[3], Yuko Kokubu[1], Thomas C. Südhof[3,4] & Marius Wernig[1,2]

Cellular differentiation and lineage commitment are considered to be robust and irreversible processes during development. Recent work has shown that mouse and human fibroblasts can be reprogrammed to a pluripotent state with a combination of four transcription factors. This raised the question of whether transcription factors could directly induce other defined somatic cell fates, and not only an undifferentiated state. We hypothesized that combinatorial expression of neural-lineage-specific transcription factors could directly convert fibroblasts into neurons. Starting from a pool of nineteen candidate genes, we identified a combination of only three factors, *Ascl1*, *Brn2* (also called *Pou3f2*) and *Myt1l*, that suffice to rapidly and efficiently convert mouse embryonic and postnatal fibroblasts into functional neurons *in vitro*. These induced neuronal (iN) cells express multiple neuron-specific proteins, generate action potentials and form functional synapses. Generation of iN cells from non-neural lineages could have important implications for studies of neural development, neurological disease modelling and regenerative medicine.

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Cellular differentiation and lineage commitment are considered to be robust and irreversible processes during development. Recent work has shown that mouse and human fibroblasts can be reprogrammed to a pluripotent state with a combination of four transcription factors. This raised the question of whether transcription factors could directly induce other defined somatic cell fates, and not only an undifferentiated state. We hypothesized that combinatorial expression of neural-lineage-specific transcription factors could directly convert fibroblasts into neurons. Starting from a pool of nineteen candidate genes, we identified a combination of only three factors, *Ascl1*, *Brn2* (also called *Pou3f2*) and *Myt1l*, that suffice to rapidly and efficiently convert mouse embryonic and postnatal fibroblasts into functional neurons *in vitro*. These induced neuronal (iN) cells express multiple neuron-specific proteins, generate action potentials and form functional synapses. Generation of iN cells from non-neural lineages could have important implications for studies of neural development, neurological disease modelling and regenerative medicine.

REGENERATIVE MEDICINE

Cell reprogramming gets direct

Cory R. Nicholas and Arnold R. Kriegstein

In a feat of biological wizardry, one type of differentiated cell has been directly converted into another, completely distinct type. Notably, the approach does not require a stem-cell intermediate stage.

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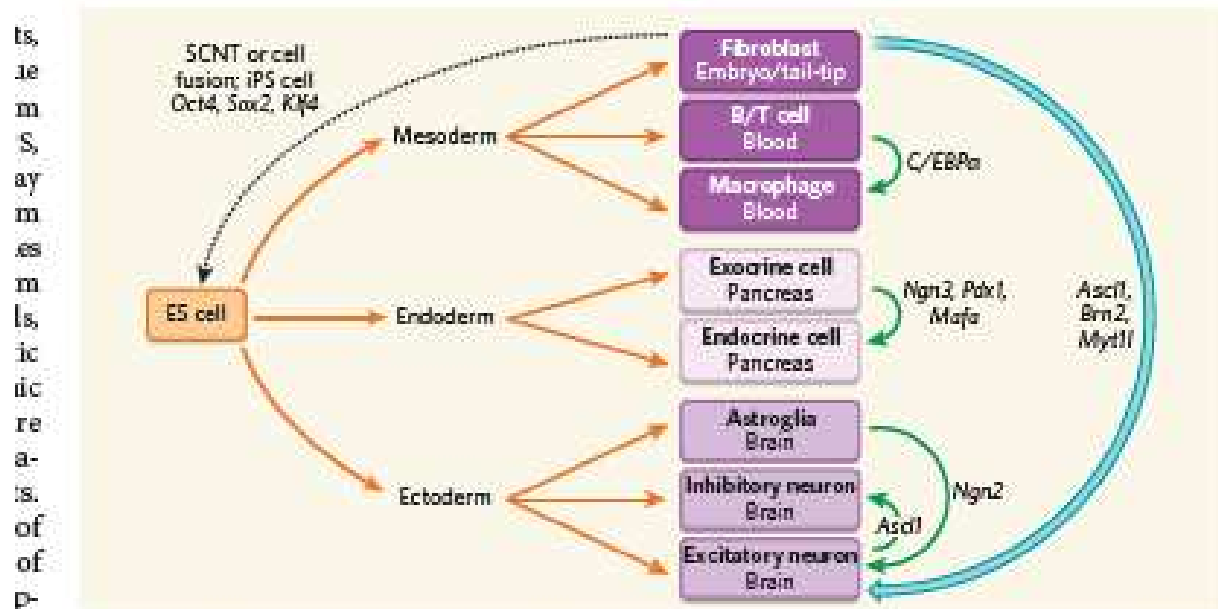
Figure 1 | Indirect and direct routes to cell-lineage reprogramming. The indirect routes involve reprogramming of a variety of adult cell types from different lineages to produce a de-differentiated embryonic stem (ES) cell state. Indirect routes (dotted arrow) include somatic-cell nuclear transfer (SCNT) or cell fusion, or creation of induced pluripotent stem (iPS) cells by the introduction of genes such as *Oct4*. But the de-differentiated cells must then be re-differentiated to adult cell types along the respective mesodermal, endodermal or ectodermal lineages. Vierbuchen *et al.*[1] demonstrate that a direct route can be taken (blue arrow): by inducing lineage-specific transcription factors encoded by genes including *Ascl1*, *Brn2* and *Myt1l*, they show that fibroblasts can be directly converted into distantly related cortical excitatory neurons. This is an advance over the intra-lineage conversion achieved between cells of the blood, pancreas or brain by induction of the other genes noted. Intra-lineage conversion studies not shown include fibroblast to macrophage and fibroblast to muscle cell by *PU.1* and *MyoD*, respectively.

Cellule staminali: breve cronistoria

-1961:“scoperte”le cs emopoietiche (ematologi)

-1981: isolate cse (ES) di topo

-1986: gene targeting su cellule ES (topi KO) (vd Nobel 2007 M.Capecchi)

-1998: prime linee cellulari di cse umane (hESC) (Thomson)

-1998: identificate cs nel cervello umano

-2006: iPS (induced pluripotent stem cells) nel topo
-2007: iPS nell’uomo (vettori virali)

-2008: Science: “Reprogramming Cells” Breakthrough of the year

-2009: “virus-free” iPS cells nell’uomo

- 2010: riprogrammazione diretta

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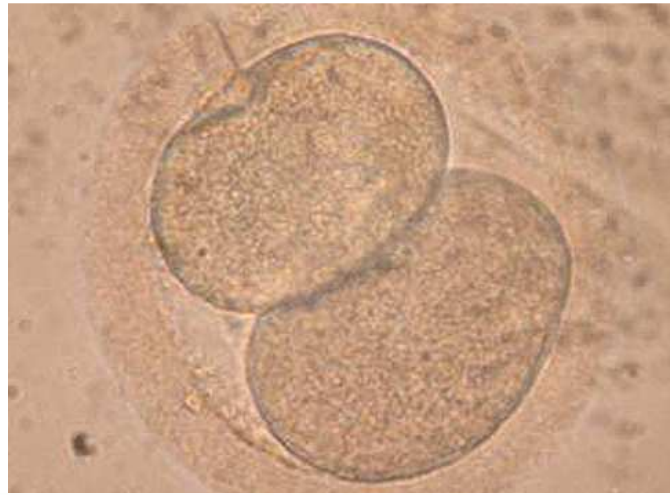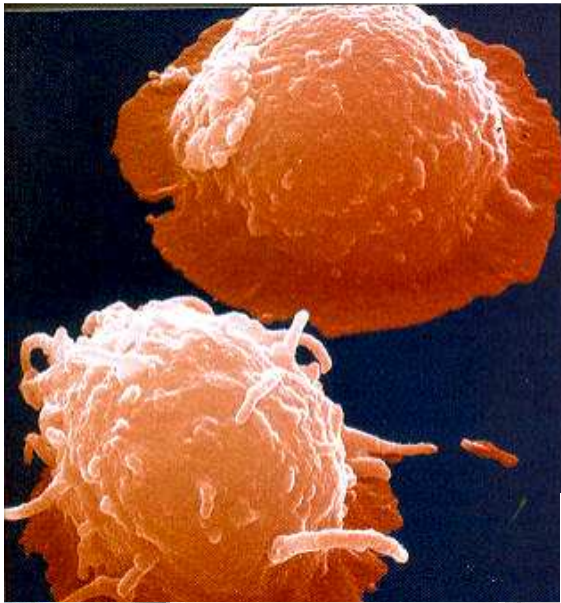
More from morulae: stem-cell lines have been created from embryos consisting of very few ce

2008

Commissioni che finanziano i propri membri, bioeticisti che sognano la «morale unica», politiche della ricerca dettate dal Vaticano. *Staminalia* racconta la storia di come un dibattito filosofico, morale e scientifico male impostato abbia finito per determinare a valanga scelte sbagliate, irrazionali, dannose. Questo accade in Italia, ma il libro è anche un resoconto appassionato e puntuale di un intero ambito cruciale ed entusiasmante della ricerca, da cui è lecito aspettarsi la rivoluzione medica del XXI secolo e che proprio per questo ha scatenato ovunque rivalità e lotte di potere basate su false contrapposizioni: come quella tra la ricerca sulle staminali embrionali, considerata inutile oltre che immorale da Bush e dal Vaticano, e le staminali adulte, cellule «etiche» che farebbero «miracoli». Peccato che il miracolismo in medicina si riveli sempre crudele verso i pazienti, che speranzosi si fanno curare prima che la ricerca abbia fatto i passi necessari. Così in nome della «sacralità dell'embrione», fondata su tesi filosofiche fragilissime, si sono moltiplicate le sofferenze umane nel mondo e ci si è inventati persino una «via italiana per la ricerca sulle staminali» (che avrebbe caratteristiche di superiore eticità perché concentrata solo sulle staminali adulte e non su quelle embrionali derivate dalla blastocisti, da alcuni considerata persona). Un caso esemplare di come non si deve, e non si può, condurre la ricerca scientifica in un paese moderno. Una vicenda che ci ha esposto al ludibrio della comunità internazionale, con articoli su «Nature» e su «Science» ovviamente ignorati in patria. Un paese dove non si può far altro che allargare le braccia e affermare: «Che ci vuole fare, stamin(it)alia».

Disegno e grafica di copertina di Guido Scarabottolo

I “miracoli” delle
cs adulte e la
demonizzazione
delle cs embrionali.
Un saggio filosofico-
scientifico che smaschera
i falsi argomenti contro
la libertà
di ricerca e che
racconta
la sperimentazione oggi
più promettente
in campo biomedico